FUNCTIONAL PROPERTIES, PHYSICOCHEMICAL INTERACTIONS, AND *IN VITRO* DIGESTIBILITY OF STARCH BLENDS AND STARCH-BLEND EXTRUDATES

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By

Tommy Z. Yuan

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Head of the Department of Food and Bioproduct Sciences
University of Saskatchewan
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Saskatoon, Saskatchewan S7N 5A8 Canada

OR

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9 Canada

ABSTRACT

Starch is one major component in many food products, the techno-functional characteristics of which are crucial for the appearance, palatability, and consumer acceptance of starchy food products. Blending and extrusion of different native starches to improve the techno-functional properties have attracted much attention recently due to the "clean-label" feature and the relatively low processing costs. In Study I, high-amylose maize starch (HA7) was blended with wheat (WHE) or potato (POT) starch at 1:1 ratio (dwb). Interestingly, the formation of a large amount of amylose-lipid complexes (ALC) was observed in HA7-WHE blend, partly accounting for its slow retrogradation during cold storage. The starch blends demonstrated good resistance to thermal degradation and thixotropic breakdown during pasting at 120°C. After cooking at 95°C, the gel strength of HA7-POT blend was higher than the theoretical value; and after cooking at 120°C, HA7-WHE and HA7-POT blends also formed gels stronger than anticipated. Importantly, it was found that reduced digestibility of cooked starches could be achieved through simple blending. Subsequently, the starch blends and three individual starches were extruded under two sets of extrusion conditions, namely "mild" and "extreme", to explore if extrusion could further diversify the functional profiles and *in vitro* digestibility. Extrusion decreased the amylose contents of starch blends and individual starches, and the mild and extreme conditions showed similar reducing effects, except for POT and HA7-POT blend. As expected, extrusion under the extreme condition degraded and/or gelatinized starches to greater extents. Starch extrudates (except for HA7 extrudates) showed instant viscosities, but their pasting viscosities were generally lower than those of their native counterparts, which could be attributed to starch gelatinization, loss of granular structure, and molecular degradation resulting from extrusion. These physicochemical changes also diminished the gelling ability of the starch samples. The information presented in this thesis will provide new directions for the food industry to produce native and pre-gelatinized starch ingredients with enhanced functional and nutritional properties.

ACKNOWLEDGMENTS

This work is dedicated to my family and friends:
It is my pleasure to have you by my side, especially at those tough moments. Thank you for your unconditional love and generous support.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of variance
ALC	Amylose-lipid complexes
C.L.	Chain length
DP	Degree of polymerization
DSC	Differential scanning calorimetry
dwb	Dry weight basis
EC	Enzyme Commission
HAMS	High-amylose maize starch
IC	Intermediate components
POT	Potato starch
RDS	Rapidly digestible starch
RS	Resistant starch
RVA	Rapid Visco Analyzer
SDS	Slowly digestible starch
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SME	Specific mechanical energy
T_c	Conclusion temperature
T_o	Onset temperature
T_p	Peak temperature
WAI	Water absorption index
WHE	Wheat starch
WSI	Water solubility index
w/v	Weight by volume
ΔH	Enthalpy change
%R	Percentage retrogradation

1 OVERVIEW

Starch is widely used in various food products as a thickener, binder, stabilizer, bulking agent, and/or gelling agent; however, the inherent undesirable features of native starch, such as low stability against shearing and high-temperature processing, and poor cold-storage stability, limit its industrial use. Although various modification approaches have been employed to improve the performance of starch ingredients (Ren, Yuan, Chigwedere, and Ai, 2021), there is an increasing demand for "clean-label" ingredients from consumers and the food industry. Blending native starches with different techno-functional characteristics can be a cost-effective and straightforward method to produce new "clean-label" starch ingredients having desirable functionality (Waterschoot, Gomand, Fierens, and Delcour, 2015).

High-amylose maize starch (HAMS) is commercially produced from maize with *amylose-extender* (*ae*) mutation, which is well recognized for the high content of resistant starch (RS) – a new type of dietary fiber (Ai and Jane, 2016). However, the undesirable functional properties of HAMS, such as low pasting viscosity and lack of a strong gelling ability under normal food processing conditions (≤ 100°C), hinder its broad applications in the food industry. In contrast, most of the common commercial waxy and normal starches show distinctly different pasting and gelling behaviors at a cooking temperature of 95°C (Liu *et al.*, 2019). Wheat starch, for example, develops a paste with relatively low viscosity upon pasting/cooking, but a good gel after storage. Potato starch, on the contrary, exhibits remarkably high pasting viscosity but forms a weak gel or only a paste.

In this thesis research, we aimed to develop new starch ingredients with diversified functional attributes and improved nutritional profiles through blending starches from different botanical origins. HAMS was blended with wheat (WHE) or potato (POT) starches at 1:1 ratio (dry weight basis; dwb), and the thermal properties, pasting and gelling behaviors over 95-140°C heating, and *in vitro* digestibility of the HAMS-WHE and HAMS-POT blends were characterized and correlated with possible physicochemical interactions between the different starches at both granular and molecular levels. To better understand the role of granular structure playing in the

unique features of the starch blends, extrusion was employed to break the granular structure. The structure-function relationships of the resulting extrudates were also characterized and compared with those of the native starch blends. The new information presented in our work will be useful for the food industry to prepare functional starch ingredients with increased RS contents that can be utilized to prepare high-quality and nutritious food products.

1.1 Objectives

a. To blend HAMS with wheat (HAMS-WHE) or potato (HAMS-POT) starch at 1:1 ratio (dry weight basis; dwb);
b. To investigate the thermal, pasting and gelling behaviors of the starch blends at heating temperatures of 95-140°C;
c. To observe the microscopic structure of starch-blend gels/pastes;
d. To determine the *in vitro* digestibility of the starch blends after cooking;
e. To break the granular structure of starch blends using a twin-screw extruder;
f. To understand the role of granular structure playing in the new functional attributes observed in the native starch blends through comparing the physicochemical properties and digestibility of starch-blend extrudates with those of the corresponding starch blends.

1.2 Hypothesis

a. Blending HAMS with WHE and HAMS with POT at 1:1 ratio (dwb) will generate new starch ingredients with the following new features:
    1) Diversified pasting profiles and gelling behaviors;
    2) Reduced tendency to retrograde;
    3) Enhanced resistant starch content;
b. The granular structure of the starches will play a key role in the functional features of the derived starch blends;
c. Extrusion will disrupt the granular structure of starches and effectively alter the physicochemical properties of the starch blends.

2 LITERATURE REVIEW

Starch, as an important energy reserve in many green plants, is found in various organs and tissues: seeds (*e.g.,* cereal grains and pulse seeds), tubers (*e.g.,* potato), roots (*e.g.,* tapioca), and fruits (*e.g.,* banana). Starches derived from different botanical sources possess various techno-functional attributes, which can function as a bulking agent, thickener, gelling agent, stabilizer, *etc.* in food and industrial products due to the high versatility and low cost (Mason, 2009). In the industry, starch is also commonly modified through chemical, physical, and/or enzymatic methods to improve its functional properties for more diverse industrial applications (Ren *et al.*, 2021). In this section, current knowledge of granular and molecular structures, functional properties, and digestibility of starch as well as their interrelationships are summarized (**Figure 2.1**).

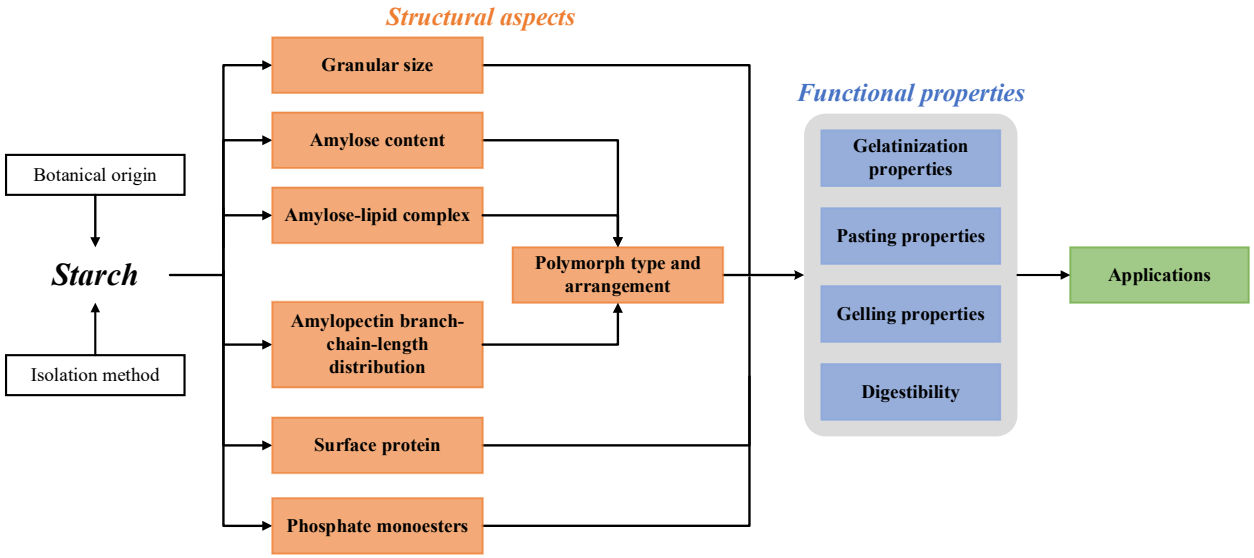


Figure 2.1 Schematic illustration of the interrelationships among the structural features and functional properties of starch.

2.1 Composition and structural features of starch

Starch is composed of three major macromolecules – amylose, amylopectin, and intermediate components (IC), all of which are homopolymers of D-glucopyranose. Amylose (**Figure 2.2 –** [a]) is essentially linear with some branching points (3-20 branches) and has a molecular weight (degree of polymerization, DP 2,300-6,300) relatively smaller than that of amylopectin (Jane, 2009). Amylopectin (**Figure 2.2 –** [b]), by contrast, has more branch linkages (~5% of all glycosidic bonds) and noticeably larger molecular weight (DP $4.3 \times 10^5 - 3.5 \times 10^7$) (Hizukuri, 1986; Yoo and Jane, 2002). IC is only present in certain starches, such as those from *amylose-extender* and *sugary-2* mutants of maize. IC usually have a highly branched structure similar to amylopectin but a molecular weight comparable to that of amylose (Jane, 2009). Starches can be categorized into waxy, normal, and high-amylose types based on the amylose content, which usually contain 1-8%, 20-40%, and >50% amylose, respectively (**Table 2.1)** (Pérez and Bertoft, 2010; Ren *et al.*, 2021).

Table 2.1 Amylose contents of common starches determined using iodine potentiometric titration.[a]

	Amylose content (%)
Waxy	
Waxy maize	1.9 ± 0.1
Normal	
Normal maize	32.0 ± 0.8
Wheat	30.7 ± 0.5
Potato	34.0 ± 0.8
Pea	39.2 ± 0.6
High-amylose	
High-amylose maize	69.7 ± 2.4

[a]Data were adapted from Liu *et al.* (2019).

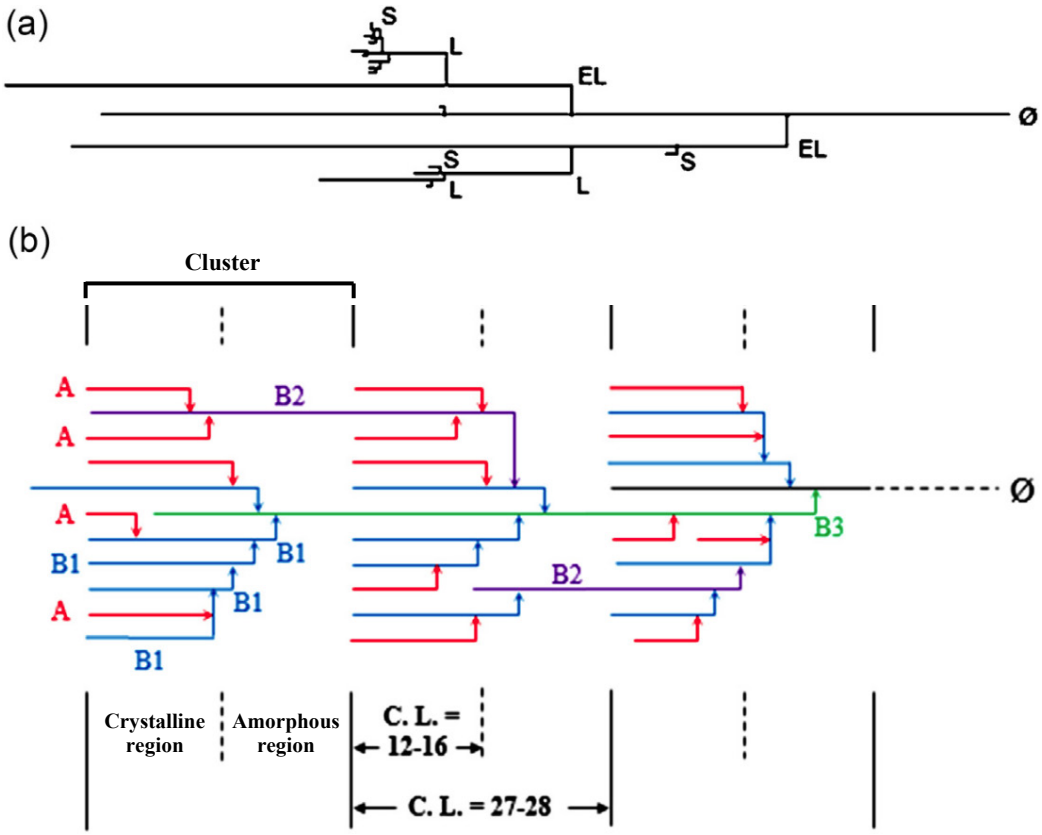


Figure 2.2 (a) Proposed molecular structure of amylose, where EL = extra long chain (DP > 2730), L = long chain (DP > 230), S = short chain (DP ~18), and Ø = reducing end. (b) Cluster model of amylopectin, where C.L. = chain length, and Ø = reducing end (Ai and Jane, 2018). Reprinted with permission from Elsevier (License has been attached in **Appendix A**).

Starch granules are insoluble in cold water due to their semi-crystalline nature, which consists of both crystalline and amorphous regions. A widely accepted model for the structure of amylopectin is the cluster model, which contains alternating crystalline and amorphous regions and has double-helical crystallites formed between two adjacent branch chains (**Figure 2.2 –** [b]). The branch linkages primarily exist in the amorphous regions as presented in the model. Amylose molecules, on the other hand, are interspersed and intertwined with amylopectin molecules in a radial orientation in granules, and amylose generally does not contribute to the crystalline structure of waxy and normal starch granules (Jane, Xu, Radosavljevic, and Seib, 1992; Kasemsuwan and Jane, 1994).

Based on the polymorphisms as revealed by X-ray diffractometry, native starches can be categorized into three types, namely A-, B-, and C-type (**Figure 2.2** – [d]**)**. The polymorphic structures reflect the different packing patterns of double-helical crystallites in native starches: a monoclinic organization for A-type, a hexagonal organization for B-type, and a combination of A- and B-type polymorphs for C-type (Pérez, Baldwin, and Gallant, 2009). The amylopectin branch-chain lengths of native starches of the three X-ray types are in ascending order of A-type < C-type < B-type (Jane *et al.*, 1999).

Low to trace concentrations of lipids, proteins, and phosphate monoesters can be found in isolated starch granules. Lipid composition in starches differs by the botanical origin but primarily consists of free fatty acids and phospholipids (Morrison, 1988). Lipids also form inclusion complex with amylose molecules, namely amylose-lipid complexes (ALC). Most ALC dissociates at a temperature of 90-125°C and shows a V-type X-ray diffraction pattern (Ai *et al.*, 2018). Proteins are also found in starch granules, and some of them possess biological functions, such as enzymes involved in starch biosynthesis (*e.g.,* granule bound starch synthase I) (Bancel, Rogniaux, Debiton, Chambon, and Branlard, 2010). Surface proteins of starch are predominately storage proteins, the content of which typically ranges from 0.06% to 0.4% (Debet and Gidley, 2006). A relatively high concentration of phosphate monoesters is found in potato starch (0.08%) (Lim, Kasemsuwan, and Jane, 1994). Each monoester phosphate group possesses two positive charges, which can lead to electrostatic repulsion between adjacent starch chains.

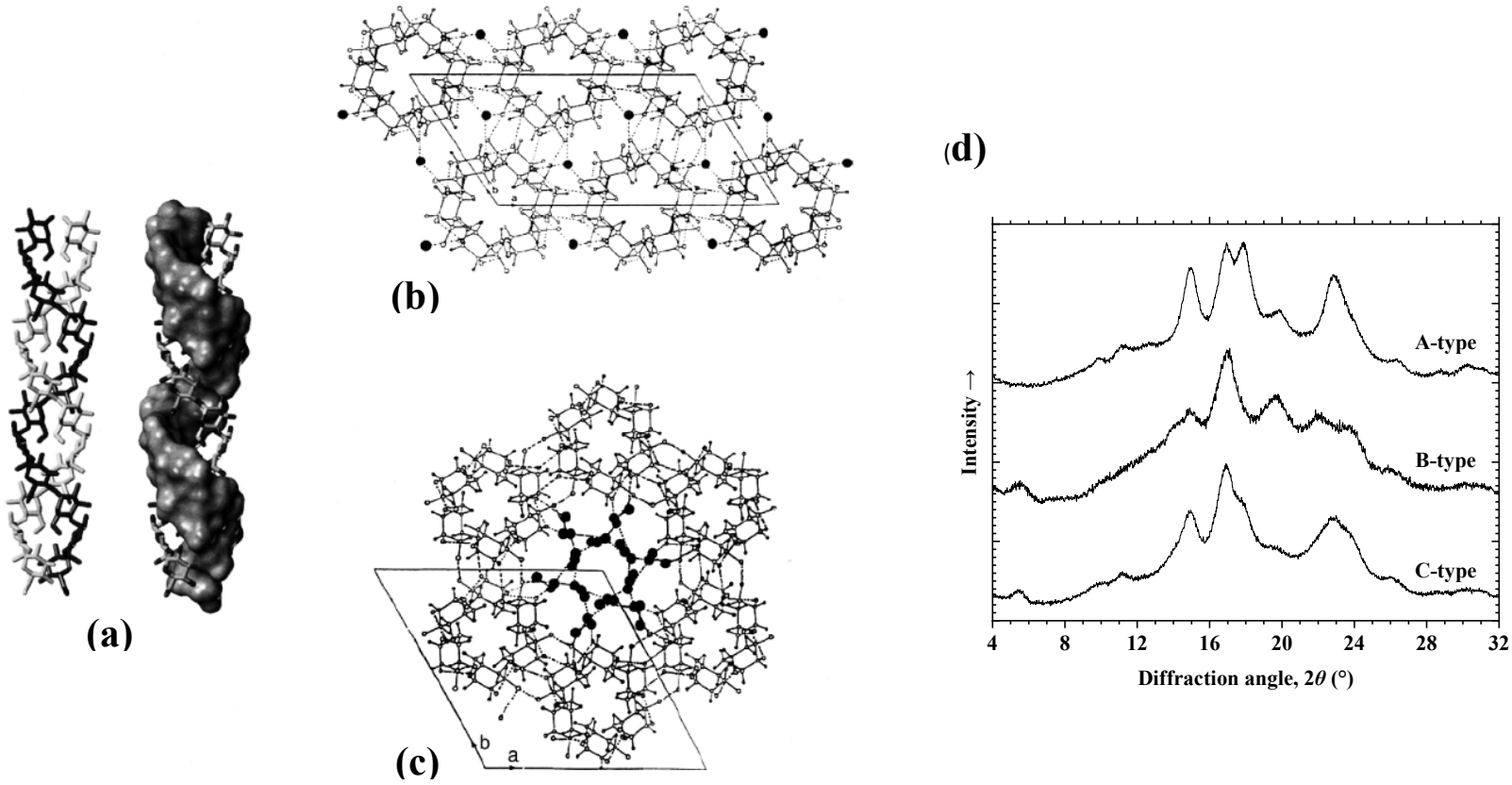


Figure 2.3 Molecular arrangement in the crystalline structure of starch (Pérez *et al.*, 2009): a) double-helical crystallite (side view) formed between two starch chains; b) monoclinic organization (top view) of double-helical crystallites in starch with the A-type X-ray diffraction pattern; c) hexagonal organization (top view) of double-helical crystallites of starch with the B-type X-ray diffraction pattern; d) A-, B-, and C-type X-ray diffraction patterns of starch. In (b) and (c), black dots (•) represent water molecules, black lines (——) represent covalent bonds, and dashed lines (----) represent hydrogen bonds. Reprinted with permission from Elsevier (License has been attached in **Appendix A**).

2.2 Functional properties of starch

2.2.1 Gelatinization and retrogradation of starch

Gelatinization temperature generally reflects the thermal stability of double-helical crystallites in starch (**Table 2.2**) (Jane *et al.*, 1999). Starch with longer amylopectin branch chains tends to possess a higher gelatinization temperature because more stable double helices are formed between branch chains. HAMS has a high gelatinization temperature partially because of its long amylopectin branch chains (Li, Jiang, Campbell, Blanco, and Jane, 2008). The amylose and IC of HAMS can also develop thermally stable double helices, which further increase its gelatinization temperature (Jiang, Campbell, Blanco, and Jane, 2010). Despite the relatively long amylopectin branch chains of potato starch, the phosphate monoesters destabilize the crystalline structure by electrostatic repulsion, leading to a low gelatinization temperature (Jane *et al.*, 1999).

Retrogradation of starch describes the change of gelatinized starch from an amorphous and solvated state to a partially crystalline and insoluble state (Gudmundsson, 1994). It can greatly affect the performance of starch during food processing and in the final products (Morris, 1990). For example, staling of bread and other starchy foods has been linked to the retrogradation of starch, and this phenomenon is detrimental to the shelf life and consumer acceptance of such starchy food products. On the other hand, rapid retrogradation of starch is a key factor to produce glass noodles with a firm texture. Jane *et al.* (1999) have demonstrated that intrinsic factors, such as amylose content, percentage of short amylopectin branch chains, and the presence of minor constituents (*e.g.,* phosphate monoesters), can affect the retrogradation of starch. For instance, gelatinized HAMS is expected to have a relatively high percentage of retrogradation (**Table 2.2**), which is ascribed to the high percentage of long branch chains of amylopectin and high amylose content. The existence of phospholipids in wheat starch and phosphate monoesters in potato starch hinders the re-formation of crystalline structure in these two starches, thus lowering their retrogradation rates (Lin and Czuchajowska, 1998; Jane *et al.*, 1999).

Table 2.2 Gelatinization and retrogradation properties of common starches[a,b]

	Gelatinization of native starch				Melting of retrograded starch				
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	%R[c]
Waxy									
Waxy maize	64.2 ± 0.2	69.2 ± 0.0	74.6 ± 0.4	15.4 ± 0.0	40.2 ± 0.5	51.3 ± 0.0	60.2 ± 0.2	7.3 ± 0.2	47.0
Normal									
Normal maize	64.1 ± 0.2	69.4 ± 0.1	74.9 ± 0.6	12.3 ± 0.0	39.0 ± 0.3	50.1 ± 0.6	59.4 ± 0.1	5.8 ± 0.5	47.6
Wheat	57.1 ± 0.3	61.6 ± 0.2	66.2 ± 0.3	10.7 ± 0.2	38.6 ± 0.3	47.7 ± 0.9	55.7 ± 0.2	3.6 ± 0.1	33.7
Potato	58.2 ± 0.1	62.6 ± 0.1	67.7 ± 0.1	15.8 ± 1.2	42.5 ± 0.4	55.7 ± 0.6	66.9 ± 1.0	7.5 ± 0.3	43.4
Pea	58.0 ± 0.1	63.5 ± 0.1	77.7 ± 0.4	13.4 ± 0.9	47.1 ± 0.5	58.1 ± 0.4	75.0 ± 1.1	6.7 ± 0.0	50.2
High-amylose									
High-amylose maize	70.6 ± 0.3	n.d.[d]	129.4 ± 2.0	16.2 ± 0.8	46.6 ± 0.5	n.d.	115.4 ± 1.8	9.9 ± 0.5	61.0

[a] Data were adapted from Jane *et al.* (1999) and Li *et al.* (2019).

[b] T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, and ΔH = enthalpy change.

[c] Percentage retrogradation (%R) = (ΔH of dissociation of retrograded starch) / (ΔH of starch gelatinization) × 100%; the gelatinized starch samples were stored at 4°C for 7 days for the retrogradation to occur.

[d] n.d.: Not detectable.

2.2.2 Pasting properties of starch

When heating in an excess amount of water, starch absorbs water and swells. Along with the resultant viscosity development, amylose and low-molecular-weight amylopectin leach out from swollen granules into the aqueous medium. The process is known as starch pasting, and the pasting behavior of starch is a major determinant of its end-use. Rapid Visco Analyzer (RVA), a rotational viscometer with heating capacity, is one of the common instruments used to determine the pasting properties of starch. During the analysis, starch is firstly suspended in an excess amount of water, and it starts to develop viscosity with continuous heating and stirring as a result of granular swelling and molecular dispersion. The viscosity change of a starch sample as a function of time and heating temperature is recorded through a typical three-stage cooking process (*i.e.,* heating, holding, and cooling) (**Figure 2.4**). After the pasting temperature is reached, the viscosity of starch rapidly increases due to the swelling of granules. The viscosity begins to decrease after the peak point because the swollen starch granules collapse. When the starch paste is cooled down, its viscosity gradually rises because of the re-association between starch remnants and leached-out molecules (especially linear amylose).

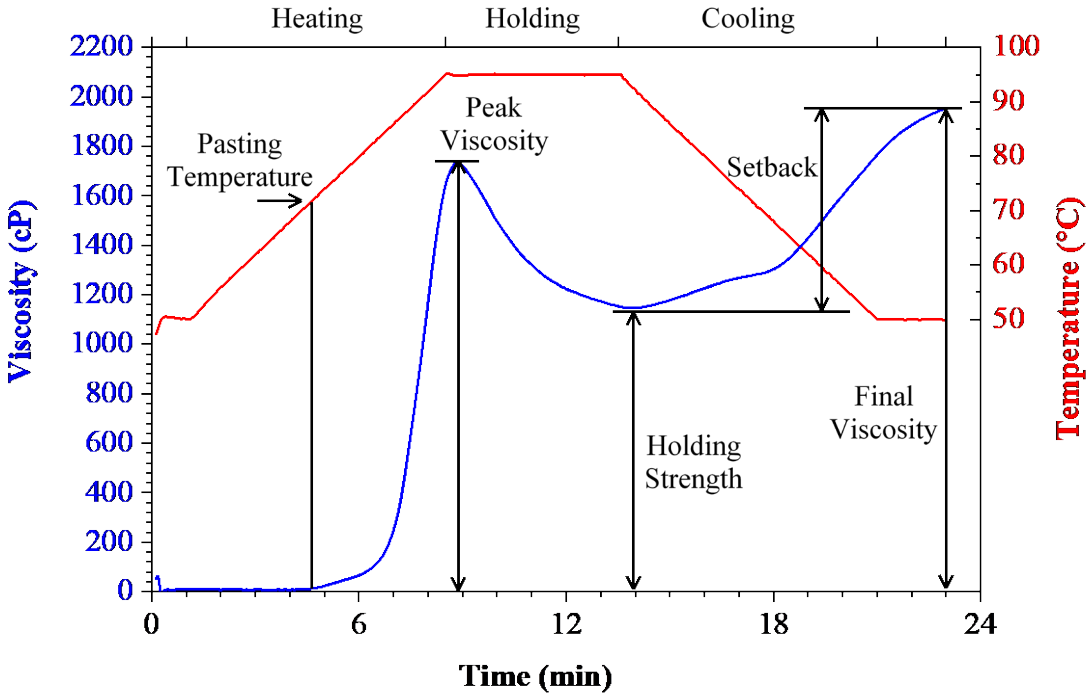


Figure 2.4 A typical pasting curve of starch (normal maize starch) (Batey, 2007).

The latest model of RVA, RVA 4800, is capable of monitoring the pasting behavior of starch up to 140°C heating temperature, which enables researchers to reveal the performance of starch and starchy food ingredients under high-temperature processing conditions, such as retorting, jet cooking, and extrusion (Liu *et al.*, 2019; Yuan, Liu, Reimer, Isaak, and Ai, 2021; Zhang, Li, You, Fang, and Li, 2020). When analyzed under high heating temperatures (> 95°C), the pasting temperatures and peak viscosities of most normal starches remained unchanged as shown by Liu *et al.* (2019) (**Figure 2.5**), while significant decreases in the holding strength and setback viscosity were observed. The authors proposed that the noted changes might be attributed to molecular breakdown and thixotropic behavior of starch molecules at high heating temperatures. However, significantly higher pasting viscosities were observed for high-amylose maize and wrinkled pea starches after pasting at 120-140°C, which was ascribed to that a greater degree of gelatinization was achieved (Liu *et al.*, 2019).

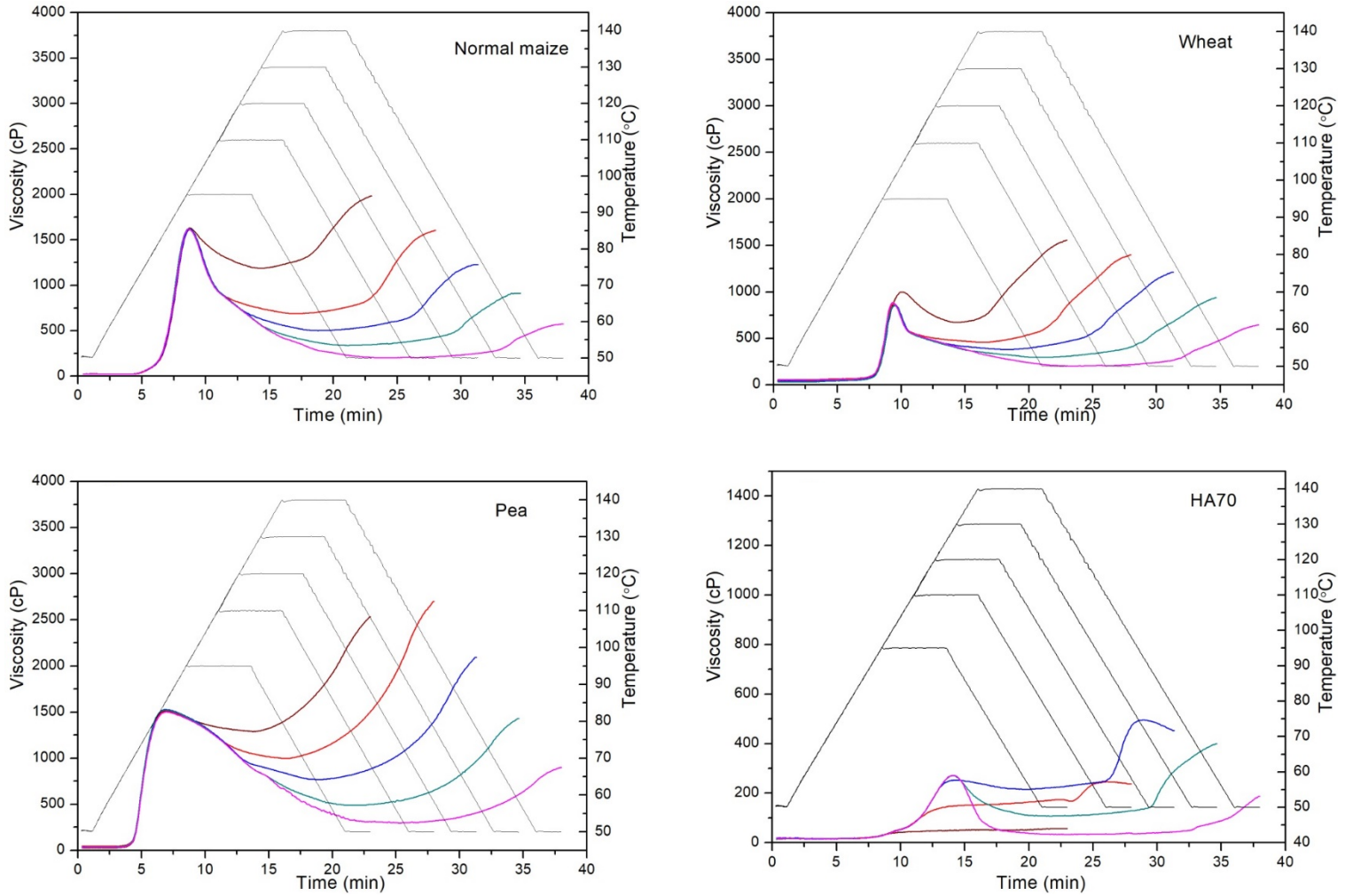


Figure 2.5 Pasting properties of common starches analyzed at heating temperatures of 95-140°C (27.5g of suspension with 7.82% solids; —: 95°C; —: 110°C; —: 120°C; —: 130°C; and —: 140°C) (Liu *et al.*, 2019).

2.2.3 Gel formation

After being gelatinized and pasted, a cooked starch paste starts to gel upon cooling. Maningat, Seib, Bassi, Woo, and Lasater (2009) have described starch gel as a composite structure, where starch granule remnants are embedded in a continuous amylose gel matrix that reinforces the structure. The textural properties of starch gels are dependent on various factors, including the deformability of gelatinized granules/remnants, the stiffness of amylose matrix, and the interactions between continuous and discontinuous phases (Maningat *et al.*, 2009). Junction zones based on the double-helical crystalline structure of starch chains are believed to be important for the gel formation of well-dispersed high-amylose starch. Klucinec and Thompson (1999) proposed that amylose and amylopectin molecules form inter- and intra- molecular double helices, which could further rearrange into crystallites; the structure could serve as the nodes to stabilize the elastic network of starch molecules. Ai, Hasjim, and Jane (2013) have demonstrated that the addition of minor components, such as lipids, could also be crucial for the formation of starch gels. The authors proposed that the developed ALC enhanced the granular integrity of starch during cooking, and the swollen starch granules, in turn, reinforced the gel network to increase the strength.

Liu *et al.* (2019) examined the gelling properties of different starches after cooking at temperatures of 95-140°C using RVA 4800 as described in **Section 2.2.2**. As the heating temperature increased, the gel hardness of normal starches progressively decreased, which could be related to the aforementioned thixotropic breakdown and thermal degradation. The scanning electron microscopy (SEM) image of the lyophilized normal maize starch gel (cooked at 95°C) revealed that the swollen starch granules/remnants (indicated by five-point stars in **Figure 2.6**) maintained their shape well and were densely packed to develop a strong gel network. However, a loosely packed, flaky structure was shown for normal maize starch after being heated at 140°C. In contrast, the gel hardness of high-amylose starches continued to increase as the heating temperature was elevated. Higher heating temperatures led to greater degrees of gelatinization of high-amylose starches, which promoted the interactions among starch molecules to develop a firm network upon cooling. Amylose molecules, as the major component in high-amylose starches, were able to re-associate and form a compact network as illustrated in the SEM image of high-amylose maize starch cooked at 140°C (**Figure 2.6**, HA70-140°C).

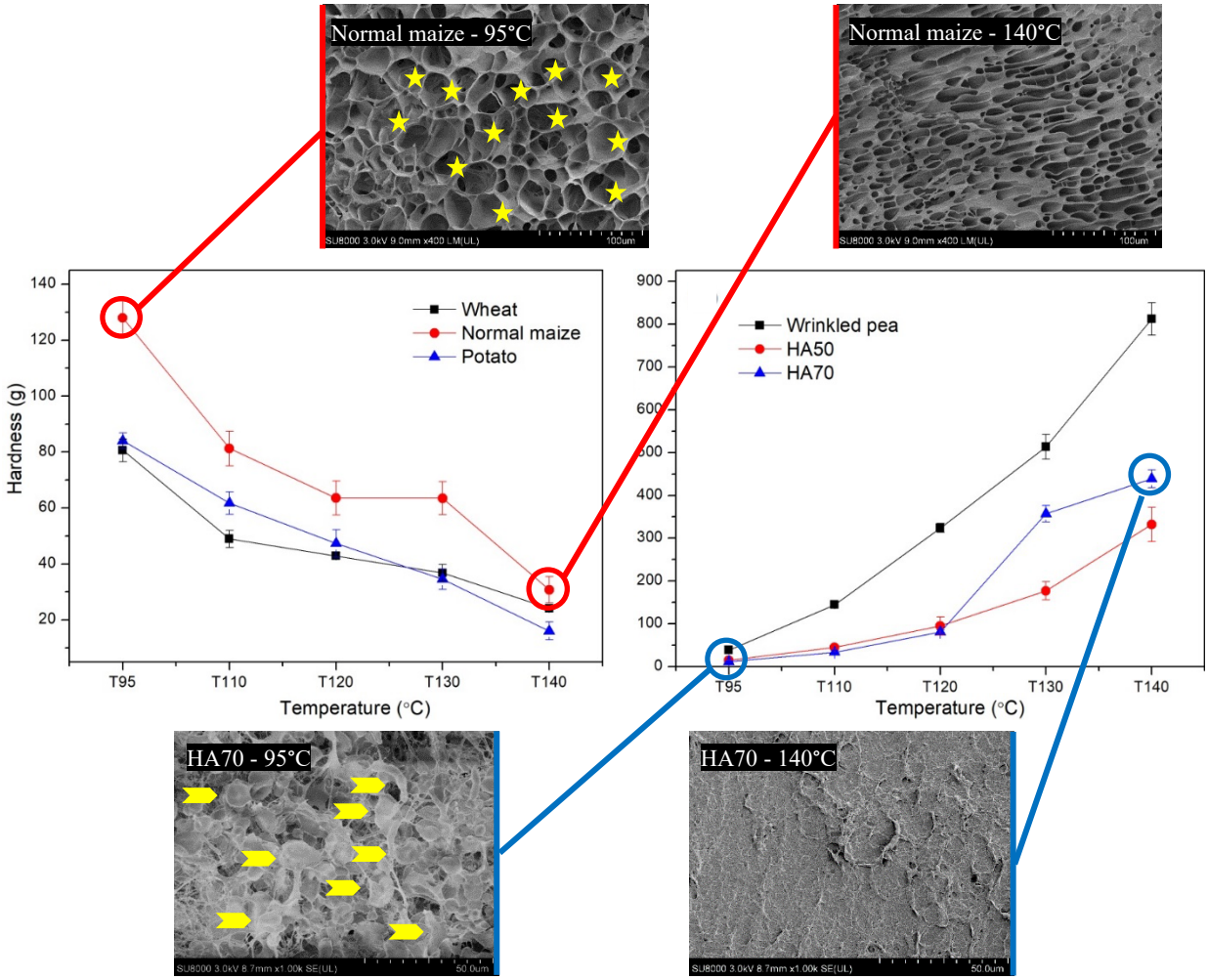


Figure 2.6 Gel hardness of normal (wheat, normal maize, potato) and high-amylose (wrinkled pea, high-amylose maize starches – HA50 and HA70) starches after pasting at 95, 110, 120, 130 and 140°C. A starch suspension of 27.5 g total weight containing 7.82% dry starch was cooked at five different temperatures and then stored at room temperature for 2.0 h for gelation to occur. Scanning electron microscopy (SEM) images of the cross-sections of lyophilized starch gels are also presented. Five-point stars mark "cells" (swollen starch granules/remnants with good integrity) found in the gel matrix, and chevrons mark starch granules partially retaining the granular morphology after cooking (Liu *et al.*, 2019).

2.2.4 Influence of minor components on starch functional properties

As mentioned in **Section 2.1**, there are low concentrations of lipids, proteins, and phosphate monoesters existing in some starches, and the presence of these components can drastically affect the functional properties of the starches. Debet *et al.* (2006) demonstrated that endogenous lipids and surface proteins could limit the swelling of wheat and maize starch granules during pasting, the removal of which lowered the pasting temperature and increased the peak viscosity of the two starches (**Figure 2.7**). Ai *et al.* (2013) showed that the formation of ALC helped to retain the integrity of starch granules during heating, and such starch granules/remnants with good integrity were essential for the formation of a firm gel from normal maize starch. Furthermore, phosphate monoesters found in some tuber starches lead to unique functional attributes (Ai *et al.*, 2018). For example, phosphate monoesters in potato starch cause repulsion between starch chains, destabilizing the crystalline structure and resulting in a low onset gelatinization temperature and remarkably high peak pasting viscosity (Jane *et al.*, 1999).

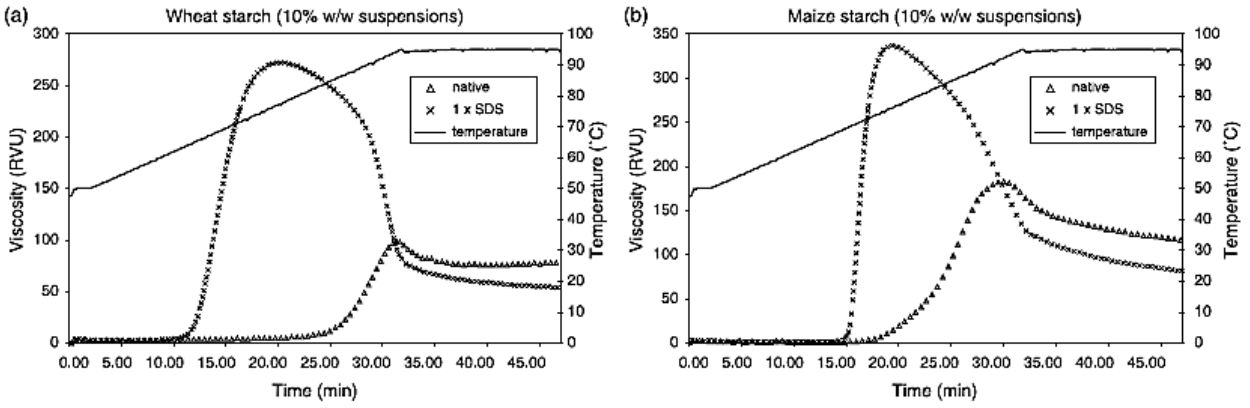


Figure 2.7 Pasting behaviors of wheat (a) and maize (b) starch before and after washing with 2% (w/v) sodium dodecyl sulfate (SDS), a detergent that can effectively remove the endogenous lipids and the surface proteins in starch granules (Debet *et al.*, 2006). Reprinted with permission from Elsevier (License has been attached in **Appendix A**).

2.3 Starch digestibility and resistant starch

Six digestive enzymes are essential for the digestion of starch in humans: salivary α-amylase, pancreatic α-amylase, glucoamylase, maltase, sucrase, and isomaltase (Lin, Lee, and Chang, 2016). Different factors, such as botanical source, amylose content, polymorphic structure and arrangement, and the formation of ALC, have been linked to starch digestibility (Zhang,

Whistler, BeMiller, and Hamaker, 2005). Resistant starch (RS), a portion of starch that cannot be digested in the small intestine and is passed into the large intestine for microbial fermentation, receives much attention due to the potential health benefits (Ai *et al.*, 2016; Birt *et al.*, 2013). Incorporating RS into foods lowers the postprandial glucose response, which can reduce the incidence of type-2 diabetes and alleviate the associated complications. It has also been shown that increased consumption of RS can be beneficial for weight management and positively affect body fat composition and distribution.

Five types of RS have been reported in the literature (Birt *et al.*, 2013; Englyst, Kingman, and Cummings, 1992). RS1 is physically inaccessible starch and usually refers to the starch entrapped in protein or cellulose matrices. RS2 is native starch with the B- or C-type polymorphic structure (Jane, Wong, and McPherson, 1997). RS3 can be produced from retrograded starch or recrystallized short-chain amylose (Cai and Shi, 2010; Zhang *et al.*, 2020). RS4 is starch chemically modified through reactions such as cross-linking or substitution (Juansang, Puttanlek, Rungsardthong, Puncha-arnon, and Uttapap, 2012; Liu, Reimer, and Ai, 2020), and RS5 is ALC (Ai *et al.*, 2013). High-amylose maize and potato starches are commercially available starch ingredients that are good sources of RS2. However, upon thermal processing, potato starch becomes readily digestible, while high-amylose maize starch retains a large proportion of RS over 95-140°C cooking (Liu *et al.*, 2020; Mishra, Monro, and Hedderley, 2008).

2.4 Starch blends and their functional properties

Recently, the growing demand for "clean-label" ingredients fosters research in physical and enzymatic modifications of starches. Blending starches with distinctly different techno-functional characteristics can be a simple and economical approach to diversify the industrial use of starches. In the past two decades, various studies have focused on blending starches with different botanical origins (Waterschoot, Gomand, Willebrords, Fierens, and Delcour, 2014), granular sizes (Gunaratne and Corke, 2007), and amylose contents (Juhász and Salgó, 2008; Park, Ibáñez, Zhong, and Shoemaker, 2007). Research efforts have also been undertaken to modify starch blends and examine the resulting effects (Gunaratne *et al.*, 2007). Altered packing of starch granules in the aqueous medium, water competition between starches, and interactions between diffused-out molecules and starch remnants play vital roles in the observed functional properties of the prepared starch blends. For example, increased gelatinization temperatures, tailored pasting

viscosity and gel hardness, and reduced syneresis have been reported for different starch blends, which clearly distinguish them from the individual starch (Waterschoot *et al.*, 2015).

Waterschoot *et al.* (2014) blended potato starch with rice or maize starch at various ratios. The peak viscosities of the starch blends were lower than expected when assuming a linear correlation between the peak viscosity and the blending ratio. The authors proposed that the phenomenon could be attributed to the reduced swelling of starch granules because of water competition. In a subsequent study, the authors examined the changes in the relative crystallinity of the individual starches and their blends during heating *in situ* using time-resolved synchrotron wide-angle X-ray diffraction (Waterschoot, Gomand, Delcour, and Goderis, 2015). The findings suggested that potato starch in the blends was gelatinized first, which efficiently bound water and reduced its availability for the other starch (*i.e.,* rice or maize) to gelatinize and paste. The limited water availability thus increased the gelatinization temperature and restricted the viscosity development of the starch blends overall.

2.5 Extrusion modification of starch

Extrusion technology is widely employed in the food and feed industries due to its versatility, low production cost, and continuous nature (Guy, 2001). Fundamentally, food extrusion is a thermomechanical process (*i.e.,* high heat and shear applied within a relatively short time) that forces the ingredients through a die under controlled conditions, and the process structures and/or puff-dries the extrudate at the end (Raiz, 2001). During extrusion, the thermomechanical energy input melts the crystallites in starch, disintegrates starch granules, and induces a series of physicochemical changes. Amylopectin is more susceptible to the degradation caused by the thermal energy and mechanical force because of its large molecular size and highly branched structure (Liu, Halley, and Gilbert, 2010).

Extrusion has been used extensively to improve the functional and nutritional properties of starch and flours. Ye *et al.* (2018) summarized that extrusion process disrupts the intra- and inter-molecular hydrogen bonds of starch and results in the exposure of more hydroxyl groups. Along with the dextrinization, extruded starch showed increased water absorption (WAI) and water solubility indices (WSI). Bryant, Kadan, Champagne, Vinyard, and Boykin (2001) extruded rice flour and observed increased cold ("instant") viscosity and decreased peak viscosity compared to its native counterpart, which could be attributed to the gelatinization and molecular degradation of

starch caused by extrusion. In recent years, studies have demonstrated that extrusion can be employed to enhance the RS contents of commercial starches (Htoon *et al.*, 2009; Shrestha *et al.*, 2015; Shrestha *et al.*, 2010; Zhang *et al.*, 2015). HAMS is particularly interesting as it largely retained the RS after extrusion. The high retention of RS in HAMS extrudate could be attributed to the following reasons: 1) Densified amorphous region in the extrudate limited or slowed down the enzymatic hydrolysis; and 2) Recrystallization of starch molecules during the digestion further decreased the susceptibility to amylolytic enzymes (Shrestha *et al.*, 2010; Zhang *et al.*, 2015).

3 PASTING AND GELATION BEHAVIORS OF HIGH-AMYLOSE MAIZE STARCH BLENDED WITH WHEAT OR POTATO STARCH EVALUATED AT HIGH HEATING TEMPERATURES

3.1 Abstract

Different approaches have been adopted to overcome the inherent shortcomings of native starches, and blending of different native starches has attracted much attention due to the "clean-label" feature and the low processing effort and cost. In the current study, we blended high-amylose maize starch (HA7) with wheat (WHE) or potato (POT) starch at 1:1 ratio (dry weight basis; dwb) and then comprehensively examined their thermal properties, pasting and gelling behaviors over the heating temperature range of 95-140°C, and *in vitro* digestibility in comparison with three individual starches. With an excess amount of water (starch:water = 1:3), gelatinization of individual starches appeared to be independent in the two starch blends. An exceptionally low percentage of retrogradation was observed for gelatinized HA7-WHE blend during cold storage, which could be partially ascribed to the formation of a large amount of amylose-lipid complexes (ALC). Greater resistance to thermal degradation and thixotropic breakdown during pasting at 120°C was found with HA7-WHE blend when compared with HA7-POT blend. Both starch blends could form gels with moderate strength (74.3-109.4 g) after cooking at 120 and 140°C. The measured resistant starch (RS) and slowly digestible starch (SDS) contents of water-boiled HA7-WHE blend were higher than the calculated values when assuming simple additivity. The interactions at both granular and molecular levels and the presence of ALC were responsible for the reported unique techno-functional attributes of HA7-WHE blend, which rendered it suitable for a broad range of food applications.

3.2 Introduction

Starch is widely used in various food products as a thickening, binding, stabilizing, bulking, and/or gelling agent; however, the inherent undesirable features of native starch, such as low resistance to breakdown caused by shear and high-temperature cooking, poor low-temperature

storage stability, and fast digestion after gelatinization, limit its industrial use. Different chemical modifications have been commonly employed to tailor the techno-functional properties of starch, which aims to widen the application spectrum of this important polysaccharide (Kaur, Ariffin, Bhat, and Karim, 2012; Ren *et al.*, 2021). Acid hydrolysis has been demonstrated to be able to reduce the pasting viscosity and manipulate the gel strength of normal maize starch (Wang, Truong, and Wang, 2003). Sangseethong, Termvejsayanon, and Sriroth (2010) have observed that the surface charge, gelatinization temperatures, and gelling behavior of tapioca starch are effectively altered by oxidation. Cross-linking can improve the stability of starch paste against shear, high-temperature processing, and acid condition (Wongsagonsup *et al.*, 2014). Studies have also shown that covalently attaching different functional groups to starch molecules, such as acetate and hydroxypropyl, improves the cold-storage stability (Pal, Singhal, and Kulkarni, 2002).

Recently, the growing demand for "clean-label" ingredients has fostered research on physical and enzymatic modifications of starch. Heat-moisture treatment, for example, is a physical modification that promotes the rearrangement of starch molecules and improves the thermal stability and gel hardness of starch (da Rosa Zavareze and Dias, 2011). Hydrolases and transferases, such as maltogenic α-amylase (EC 3.2.1.133), pullulanase (EC 3.2.1.41), isoamylase (EC 3.2.1.68), and 4-α-glucanotransferase (4αGT; EC 2.4.1.25), have been utilized to produce starch-based ingredients with diversified functional properties and enhanced nutritional value (Li, Li, Zhu, and Ai, 2021; Li, Yuan, and Ai, 2020; Liu, Gu, Hong, Cheng, and Li, 2017; van der Maarel and Leemhuis, 2013). As examples, treatment with maltogenic α-amylase considerably retards retrogradation, reduces pasting viscosities, and enhances enzymatic resistance of starch (Li *et al.*, 2021). The sequential use of enzymatic debranching (either by isoamylase or pullulanase) and physical recrystallization can generate resistant starch (RS) ingredients with exceptional thermal stability (Li *et al.*, 2020; Liu *et al.*, 2017). Thermoreversible starch gel that possesses functional attributes similar to those of gelatin can be produced through 4αGT modification (van der Maarel *et al.*, 2013).

Blending different starches is another "clean-label" approach that is economical to prepare starch ingredients with a target functionality. Waterschoot *et al.* (2015) blended B-type potato starch with one of the selected A-type cereal starches (*i.e.,* normal maize, waxy maize, rice, or waxy rice) and determined the thermal properties of the individual starches and their blends with limited water (starch dry matter:water = 1:1). The authors reported that potato starch was

gelatinized at a temperature lower than the other starch in the blends and that the blending with potato starch considerably increased the peak gelatinization temperature (T_p) of the other starch. The phenomenon could be ascribed to water competition between potato and the other starch during heating. Potato starch was gelatinized first to more effectively bind with water, which left less free water available to the other starch present, thus resulting in an increase in its T_p. Gunaratne *et al.* (2007) blended potato starch with amaranth starch at 1:1 ratio (dry weight basis; dwb) and observed that the blend displayed peak and breakdown viscosities lower than those of potato starch. A shoulder before the main peak of the pasting profile of the blend was also noted, which could be attributed to that the leached-out amylose from potato starch restricted the swelling of amaranth starch granules as explained by the authors. The physicochemical properties of various starch blends have been reviewed and related to the chemical compositions and structures of the included starches (Waterschoot *et al.*, 2015).

High-amylose maize starch (HAMS) is commercially produced from maize with *amylose extender* (*ae*) mutation, which is well recognized for the high content of RS – a new type of dietary fiber (Ai *et al.*, 2016). Nevertheless, under normal food processing conditions (*e.g.*, a typical cooking temperature ≤ 100°C), HAMS – due to the high gelatinization temperatures (conclusion temperature, T_c > 100°C) – develops little viscosity and fails to form a good gel or film (Ai *et al.*, 2013; Zhang *et al.*, 2020). To gain a greater understanding of the performance of HAMS in a broader application spectrum, Rapid Visco Analyzer 4800 (RVA 4800), the newest model of the instrument having high-temperature capability, was used to characterize the physicochemical properties of HAMS in a heating temperature range of 95-140°C (Liu *et al.*, 2019). As the heating temperature increased from 95 to 140°C, HAMS became fully gelatinized and thus could provide substantially higher pasting viscosity and stronger gelling ability. In contrast, most waxy and normal starches showed distinctly different pasting and gelling behaviors over the cooking temperatures of 95 to 140°C in the same study. As examples, at the heating temperature of 95°C, wheat starch displayed the lowest pasting viscosity, whereas potato starch exhibited the highest pasting viscosity among the tested normal starches (Liu *et al.*, 2019); and both starches formed a good gel. When the heating temperature was progressively elevated to 140°C, the pasting viscosities and gelling capability of potato and wheat starches considerably decreased. Based on the understanding of the distinct intrinsic properties of these native starches, we hypothesized that

blending HAMS with wheat or potato starch at 1:1 ratio will generate new starch ingredients possessing diversified functional features and increased RS contents.

Therefore, in the present study, HAMS was blended with wheat (WHE) or potato (POT) starch at 1:1 ratio (dwb), respectively, and the HAMS-WHE and HAMS-POT blends and the three individual starches were characterized for thermal properties, pasting and gelling behaviors in a heating temperature range of 95-140°C, as well as *in vitro* digestibility after cooking. This research revealed the unique physicochemical properties of the starch blends from various botanical sources and their interrelationships with the granular and molecular interactions between the different starches. The determination of pasting and gelling properties at high heating temperatures up to 140°C was particularly important because HAMS could be completely gelatinized and well dispersed, which thus could have more effective molecular interactions with the other two starches. The new information as presented in our work will be useful for the food industry to enhance the functionality of HAMS to deliver RS in food products.

3.3 Materials and Methods

3.3.1 Materials

High-amylose maize starch (HA7; AmyloGel™ 03003; purity ≥ 98.5%) and potato starch (POT; C*Gel™ 3002; purity ≥ 96.7%) were donated by Cargill Inc. (Minneapolis, MN, U.S.A.). Wheat starch (WHE; Midsol™ 50; purity ≥ 96.6%) was a gift from MGP Ingredients Inc. (Atchison, KS, U.S.A.). The amylose contents of HA7, POT, and WHE were 63.6%, 24.8%, and 35.2%, respectively, as determined using an iodine colorimetric method of Chrastil (1987). HA7-WHE and HA7-POT blends were prepared at 1:1 ratio on a dry weight basis (dwb). After being accurately weighed, the starch blends were sealed in screw-capped containers and vigorously shaken for about 20 min to achieve homogeneous mixing. Porcine pancreatin and amyloglucosidase were purchased from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). D-Glucose Assay Kit was procured from Megazyme Ltd. (Co. Wicklow, Ireland). All the other chemicals used in the study were ACS grade and acquired from Fisher Scientific Co. (Ottawa, ON, Canada).

3.3.2 Thermal properties of starch

Thermal properties of the three native starches and the starch blends were analyzed using a differential scanning calorimeter (DSC 8000, PerkinElmer Inc., Waltham, MA, U.S.A) following the method of Song and Jane (2000). Approximately 10 mg of the sample was accurately weighed, wet with three volumes of distilled water, and sealed in a stainless-steel pan (PerkinElmer Inc.). After equilibrating at ambient temperature for more than 2 h, the sample was scanned from 10 to 160°C at a rate of 10°C/min. After immediate cooling to 10°C at a rate of 20°C/min, a rescan from 10 to 160°C at 10°C/min was performed to examine the dissociation of the formed amylose-lipid complexes (ALC) (Hasjim *et al.*, 2010). After the gelatinization step, the sample pan was stored at 4°C for 7 days, followed by heating using the same procedure to determine the retrogradation property of the starch. Onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy change (ΔH) of the thermal transitions were analyzed using Pyris™ Thermal Analysis (Version 13.3.1.0014, PerkinElmer Inc.). The percentage of retrogradation of the sample was calculated in accordance with the following equation:

%Retrogradation = (ΔH of melting of retrograded starch) / (ΔH of gelatinization of native starch) × 100%

Theoretical ΔH values of the dissociation of ALC in the immediate rescan of the starch blends were calculated using the following equation:

Theoretical ΔH of ALC dissociation of starch blend = (ΔH of ALC dissociation of Starch A × 50%) + (ΔH of ALC dissociation of Starch B × 50%)

Theoretical percentages of retrogradation of the starch blends were calculated using the following equation:

Theoretical %Retrogradation of starch blend = (%Retrogradation of Starch A × 50%) + (%Retrogradation of Starch B × 50%)

3.3.3 Pasting and gelling properties of starch

Pasting properties of the three native starches and the starch blends were determined using a Rapid Visco-Analyzer (RVA 4800, Perten Instruments Australia Pty Ltd., Sydney, Australia) following the method of Liu *et al.* (2019) with minor changes. In brief, the prepared starch slurry (8.0% dry solids, w/w; 28.0 g in total) was equilibrated at 50°C for 1 min, heated to the target holding temperature (95, 120, or 140°C) at a rate of 6°C/min, maintained at the temperature for 5

min, cooled to 50°C at 6°C/min, and finally held at 50°C for 2 min. Pasting temperature, peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, peak time, and peak temperature of the starch were recorded.

After cooking by RVA 4800 at 95, 120, or 140°C, the resulting starch paste was transferred into a screw-capped plastic canister (internal diameter = 33.0 mm) to achieve a height of approximately 25 mm. The container was capped and kept at ambient temperature (~22°C) for 2.0 h for the cooked starch to gel. The strength of the obtained starch gel was measured using a texture analyzer equipped with a TA-10 cylindrical probe (diameter = 12.7 mm; TA.XT. Plus, Texture Technologies Corp., South Hamilton, MA, U.S.A.). When a trigger force of 0.5 g was attained, the probe proceeded to penetrate the gel at a test speed of 0.5 mm/s and a compression depth of 10.0 mm. The maximum positive force determined during the compression was recorded as the gel strength. Theoretical gel strength of the starch blends was calculated using the following equation:

Theoretical gel strength of starch blend = (Gel strength of Starch A × 50%) + (Gel strength of Starch B × 50%).

3.3.4 Microstructure of starch gel

Gels were prepared from the three native starches and the starch blends after cooking by RVA 4800 at 95, 120, or 140°C as described in **Section 2.3**. The generated starch gel was instantly frozen using liquid nitrogen (approximately -196°C) and then lyophilized at -25°C for 48 h. The lyophilized starch gel was sputter coated with gold and then observed using a field-emission scanning electron microscope (SEM, SU8010, Hitachi High Technologies Canada Inc., Rexdale, ON, Canada) under the conditions of 5.0 kV and 10 μA to reveal the microstructure. Representative images of the cross section of the freeze-dried starch gel were taken at 1,500× magnification.

3.3.5 *In vitro* digestibility of cooked starch

In vitro digestibility of the three native starches and the starch blends upon cooking at boiling water temperature was analyzed using the method of Englyst *et al.* (1992) with slight modifications (Li *et al.*, 2019). The sample containing 600 mg of starch was suspended in 15.0 mL of distilled water and then cooked in a boiling water bath for exactly 10.0 min, followed by cooling to room temperature. After the addition of 5.0 mL sodium acetate buffer [pH 5.2, 0.4 M, containing

0.18% (w/v) calcium chloride and 0.08% (w/v) sodium azide], the sample tube was equilibrated in a shaking water bath (37°C, 160 rpm) for around 15 min. An aliquot (5.0 mL) of freshly prepared enzyme cocktail of porcine pancreatin extract and amyloglucosidase was added to hydrolyze the starch in the same water bath. The amounts of glucose liberated from the amylolysis at 20 and 120 min were quantitated using Megazyme D-glucose Assay Kit. The contents of rapidly digestible starch (RDS; digested within 20 min), slowly digestible starch (SDS; digested between 20 and 120 min), and resistant starch (RS; undigested after 120 min) were calculated according to the equations described by Englyst *et al.* (1992). Theoretical RDS/SDS/RS contents of the cooked starch blends were calculated using the following equation:

Theoretical %RDS/SDS/RS of cooked starch blend = (%RDS/SDS/RS of cooked Starch A × 50%) + (%RDS/SDS/RS of cooked Starch B × 50%)

3.3.6 Statistical analysis

Each sample was analyzed in triplicate for all the tests. Statistical differences among the three native starches and the starch blends were evaluated using one-way ANOVA, Tukey's multiple comparison test at a significance level of 0.05. The statistical evaluation was conducted using IBM SPSS Statistics Analysis (Version 27.0).

3.4 Results and Discussion

3.4.1 Thermal properties of starch

Native HA7 showed gelatinization temperatures significantly higher than those of native WHE and POT (**Table 3.1**), which was attributed to long-chain double-helical crystallites developed from amylose, intermediate components, and/or long branch chains of amylopectin in HA7 (Jane *et al.*, 1999; Jiang *et al.*, 2010). POT demonstrated the highest gelatinization ΔH (20.3 J/g), followed by HA7 (16.2 J/g) and WHE (12.8 J/g). The immediate rescan of the three starches after gelatinization revealed that ALC were present in HA7 (dissociation ΔH = 1.9 J/g) and WHE (dissociation ΔH = 1.0 J/g) but not in POT, in good accordance with the findings reported in previous literature (Liu *et al.*, 2019).

The gelatinization temperatures of the starch blends, in general, reflected the features of individual starches (**Table 3.1** and **Figure 3.1**): HA7-WHE and HA7-POT blends showed T_o and T_p close to those of WHE and POT, respectively, and T_c almost identical to that of HA7. The

observation differed from the data reported by Waterschoot *et al.* (2015) because an excess amount of water (three volumes) was used in this study and the two starches in each blend did not compete for water during DSC scanning. Interestingly, with respect to the formation of ALC, the determined ΔH of ALC dissociation in the HA7-WHE blend (1.9 J/g) was greater than the calculated value (1.5 J/g), suggesting that the amount of ALC developed in this blend was larger than anticipated. However, the measured ΔH value of HA7-POT blend (0.7 J/g) was smaller than the calculated value (1.0 J/g), suggesting that the formation of ALC in this blend was less than expected. The larger amount of ALC found in HA7-WHE blend than the theoretical value could be attributed to that amylose molecules leaching out from HA7 after the first scan complexed with excess endogenous lipids existing on the surface of WHE granules during cooling (Baszczak, Fornal, Amarowicz, and Pegg, 2003). In contrast, the amount of endogenous lipids in POT was extremely low (~0.2%) (Baszczak *et al.*, 2003), and thus no ALC formation was observed in POT itself nor did the blending enhance the formation of such single-helical complexes in HA7-POT sample.

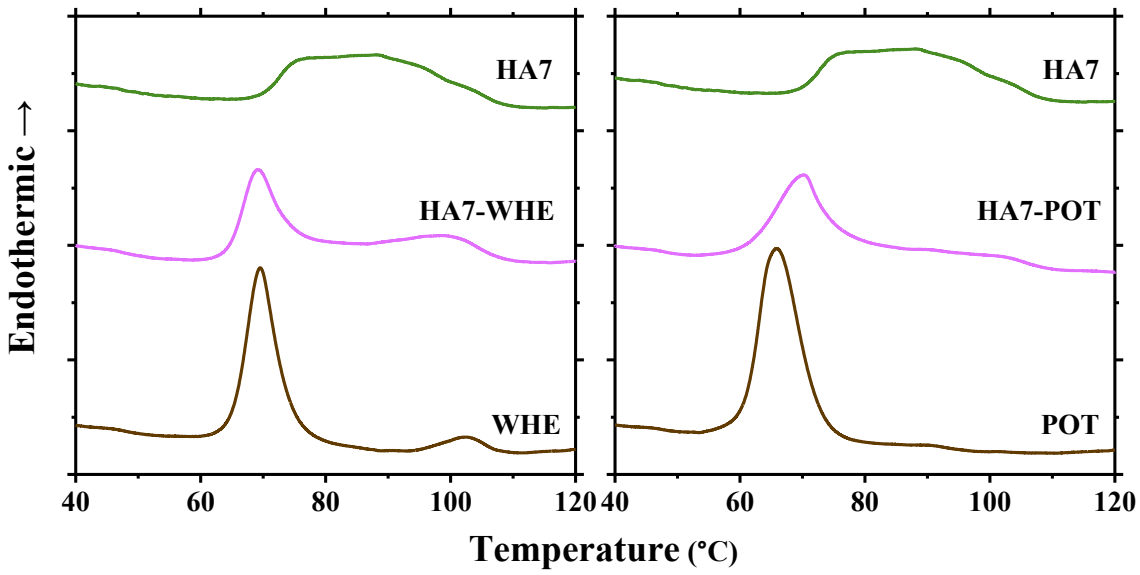


Figure 3.1 Differential scanning calorimetry (DSC) thermograms of three native starches and starch blends at 1:1 ratio (dwb) in the first scan.

Table 3.1 Thermal properties of three native starches and starch blends at 1:1 ratio (dwb). [a,b]

	First scan (starch gelatinization)				Immediate rescan (dissociation of amylose-lipid complexes)			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
HA7	70.7 ± 0.3 c	86.7 ± 1.4 c	107.2 ± 0.6 b	16.2 ± 1.2 b	88.4 ± 0.3 a	101.1 ± 0.0 a	107.3 ± 0.7 ab	1.9 ± 0.1 b
HA7-WHE	64.9 ± 0.1 b	69.3 ± 0.1 b	107.7 ± 1.1 b	14.4 ± 0.0 ab	93.5 ± 0.9 ab	100.8 ± 0.3 a	106.1 ± 0.2 a	1.9 ± 0.2 b (1.5)[c]
WHE	64.8 ± 0.5 b	69.1 ± 0.6 b	73.8 ± 0.6 a	12.8 ± 0.6 a	98.4 ± 2.8 b	105.4 ± 0.4 c	108.5 ± 0.7 b	1.0 ± 0.1 a
HA7-POT	61.6 ± 0.4 a	69.9 ± 0.3 b	106.4 ± 0.3 b	13.7 ± 0.8 a	90.6 ± 2.7 a	101.9 ± 0.3 b	107.3 ± 0.3 ab	0.7 ± 0.0 a (1.0)
POT	60.9 ± 0.0 a	66.1 ± 0.2 a	72.9 ± 0.3 a	20.3 ± 0.2 c	n.d.[d]	n.d.	n.d.	n.d.

[a] Determined using a differential scanning calorimeter (DSC). T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature, and ΔH: enthalpy change.

[b] Values are presented as average ± standard deviation (n = 3). The numbers with the same letter in one column are not significantly different at $p < 0.05$.

[c] Theoretical ΔH of ALC dissociation of starch blend = (ΔH of ALC dissociation of Starch A × 50%) + (ΔH of ALC dissociation of Starch B × 50%)

[d] n.d.: not detectable.

Among the three native starches, HA7 exhibited the highest percentage of retrogradation (44.8%), followed by POT (20.9%) and WHE (10.6%) (**Table 3.2**). The fastest retrogradation rate of HA7 resulted from its highest amylose content (63.6%) and the presence of more long branch chains of amylopectin and intermediate components (Jane *et al.*, 1999). By contrast, the occurrence of more short branch chains of amylopectin might contribute to the slowest retrogradation rate of WHE (Jane *et al.*, 1999). Intriguingly, HA7-WHE blend showed a percentage of retrogradation comparable to that of WHE (9.2% *versus* 10.6%), which was substantially lower than the theoretical value (27.7%). In contrast, the measured value of HA7-POT blend was higher than the corresponding theoretical data (44.9% *versus* 32.9%). The substantial inhibition of retrogradation in HA7-WHE blend could be primarily ascribed to the formation of a noticeable amount of ALC as discussed above (**Table 3.1**). It is a well-known fact that the development of single-helical ALC in starch can effectively retard starch retrogradation, a process involving the re-association of adjacent starch chains to partially restore the double-helical crystalline structure (Mariscal-Moreno, Figueroa-Cárdenas, Santiago-Ramos, and Rayas-Duarte, 2019; Zhou, Robards, Helliwell, and Blanchard, 2007). Since retrogradation is the main factor responsible for syneresis and other related instability phenomena of cooked starch during storage, particularly under cold conditions (*e.g.*, 4°C), the significant reduction in retrogradation by simply blending HA7 and WHE could be utilized to prepare native starch ingredients with improved storage stability.

Table 3.2 Melting behaviors of retrograded starches and starch blends at 1:1 ratio (dwb). [a,b]

	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	%Retrogradation[c,d]
HA7	47.4 ± 2.5 bc	80.2 ± 1.6 d	109.7 ± 1.0 d	7.3 ± 0.7 d	44.8 ± 0.8 c
HA7-WHE	42.8 ± 1.5 ab	55.2 ± 0.3 a	61.9 ± 0.9 a	1.3 ± 0.2 a	9.2 ± 1.4 a (27.7)[e]
WHE	39.3 ± 0.2 a	56.1 ± 0.6 a	64.8 ± 2.6 a	1.4 ± 0.1 a	10.6 ± 0.5 a
HA7-POT	44.1 ± 0.2 bc	61.4 ± 1.4 b	93.8 ± 4.6 c	6.2 ± 0.5 c	44.9 ± 3.4 c (32.9)
POT	48.1 ± 2.6 c	65.4 ± 0.3 c	74.2 ± 0.3 b	4.2 ± 0.2 b	20.9 ± 0.8 b

[a] Determined using a differential scanning calorimeter. T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature, and ΔH: enthalpy change.

[b] Values are presented as average ± standard deviation (n = 3). The numbers with the same letter in one column are not significantly different at $p < 0.05$.

[c] %Retrogradation = (ΔH of melting of retrograded starch) / (ΔH of gelatinization of native starch) × 100%.

[d] ΔH of gelatinization of native starch used in the calculation has been reported in **Table 3.1**.

[e] Theoretical %Retrogradation of starch blend = (%Retrogradation of Starch A × 50%) + (%Retrogradation of Starch B × 50%)

3.4.2 Pasting properties of starch

When analyzed at the holding temperature of 95°C, POT showed the highest peak viscosity (9081.0 cP), followed by WHE (1345.5 cP) and HA7 (40.5 cP) (**Figure 3.2** and **Table 3.3**). The remarkably higher peak viscosity of POT resulted from the electrostatic repulsion between negatively charged phosphate monoester groups on amylopectin molecules and the large granular size (Lim *et al.*, 1994; Shi and BeMiller, 2002). The considerably higher pasting temperature and lower peak viscosity of WHE were related to the formation of ALC in this cereal starch (**Table 3.1**) (Debet *et al.*, 2006; Jane *et al.*, 1999). The pasting at 95°C was unable to completely gelatinize HA7 (**Table 3.1**), and thus the starch granules swelled to a low extent and developed little viscosity.

As the holding temperature increased from 95 to 120 and 140°C, POT demonstrated similar peak viscosity, while that of WHE decreased. The decrease in the peak viscosity of WHE at 120 and 140°C could be explained by that the two high heating temperatures melted ALC in WHE and therefore the granules could not swell to the same level as at 95°C (Liu *et al.*, 2019). The pasting at 120 and 140°C noticeably reduced the trough and final viscosities of WHE and POT, resulting from thermal degradation and thixotropic breakdown as explained by Liu *et al.* (2019). The heating at 120 and 140°C markedly elevated the pasting viscosities of HA7 because higher degrees of starch gelatinization were attained at the two temperatures above 95°C. Moreover, HA7 exhibited no breakdown viscosity at 120°C and the pasting viscosity gradually increased toward the end of the pasting curve; however, at 140°C, HA7 displayed a breakdown viscosity of 191.0 cP (**Figure 3.2** and **Table S-1**). Therefore, in the "post-peak" region, the pasting viscosity of HA7 was notably higher at 120°C than at 140°C. The pasting behaviors of all the three native starches at 95-140°C are in good agreement with the findings reported by other researchers (Liu *et al.*, 2019; Zhang *et al.*, 2020).

The pasting viscosity development of HA7-WHE and HA7-POT blends over the heating temperatures of 95-140°C could not be simply interpreted as a proportional superposition of the pasting performance of the individual starches (**Figure 2** and **Table S-1**). As the heating temperature increased from 95 to 140°C, the pasting profile of HA7-POT blend changed in a pattern similar to those of POT and other waxy and normal starches (except for WHE): the pasting temperature and the peak viscosity and time generally remained the same, the trough and final viscosity gradually decreased, and the breakdown viscosity progressively increased (Liu *et al.*,

2019). By contrast, among the three heating temperatures, HA7-WHE exhibited the highest peak, trough, and final viscosities but the smallest breakdown viscosity at 120°C. The different change patterns in the pasting profiles of HA7-WHE and HA7-POT blends could be related to the significance of HA7 for the viscosity development of the blends. In HA7-POT blend, POT was the major contributor to the pasting viscosities at the three different holding temperatures because its pasting viscosities substantially exceeded those of HA7. Consequently, the pasting profile of HA7-POT blend changed in a pattern similar to that of POT when the heating temperature was elevated from 95 to 140°C. However, the differences in the peak, trough, and final viscosities between HA7 and WHE were considerably lower than those between HA7 and POT at 120 and 140°C. Thus, the viscosity development of HA7 at those two heating temperatures could effectively influence the pasting profile of HA7-WHE blend. This explanation was supported by the observation that the peak time of HA7-WHE sample was elevated from 8.9 min at 95°C to 13.0 min at 120 and 140°C, which was the peak time of HA7 at the two high heating temperatures (**Figure 3.2** and **Table 3.3**), suggesting that the peak viscosity development of HA7 significantly contributed to the peak value of HA7-WHE blend. It is noteworthy that the pasting viscosity of HA7-WHE was even higher than that of WHE alone between 12.9 and 29.6 min at 120°C heating, which suggested that certain physical interactions occurred among the different components from those two starches to promote the viscosity development. The comparison among the pasting profiles of HA7-WHE sample at the three heating temperatures also indicated that this starch blend was resistant to the pasting at 120°C, which is a typical heating temperature during canning, retorting, and jet-cooking. Therefore, the HA7-WHE blend could be used as a thickener, binder, and/or stabilizer with desirable resistance against the harsh processing conditions in those applications. However, it is important to point out that the HA7-WHE sample still possessed lower pasting viscosities than those of HA7-POT at 95-140°C, primarily because of the presence of POT in the latter.

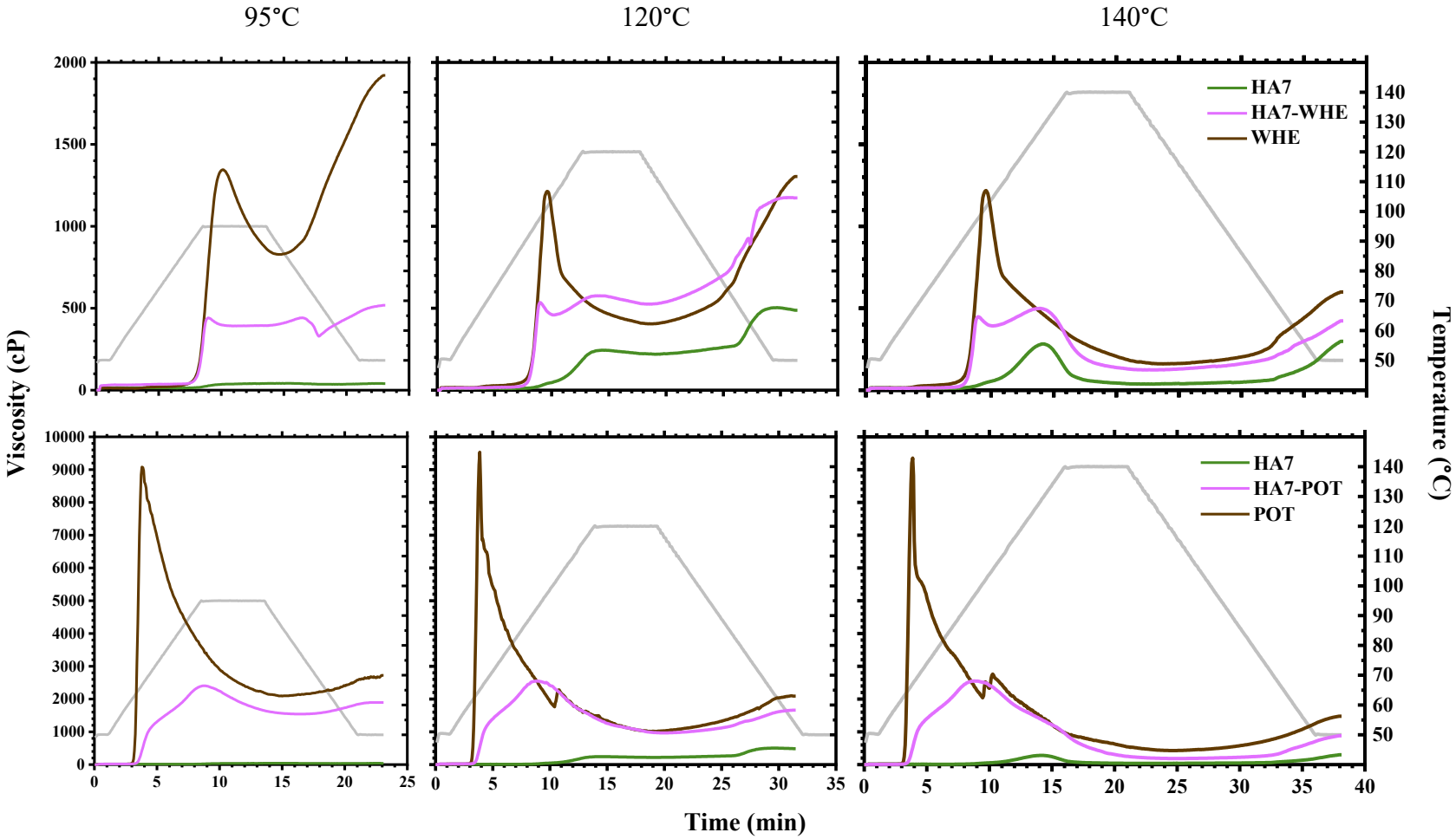


Figure 3.2 Pasting behaviors of three native starches and starch blends at 1:1 ratio (dwb) analyzed using RVA 4800 at different holding temperatures. Starch suspensions (28.0 g in total) containing 8.0% of dry solids were used for the analysis.

Table 3.3 Pasting parameters of three native starches and starch blends at 1:1 ratio (dwb) analyzed using RVA 4800 at heating temperatures of 95, 120, and 140°C.[a,b]

Starch	Holding temperature (°C)	Viscosity (cP)					Peak time (min)	Pasting temperature (°C)
		Peak	Trough	Breakdown	Final	Setback		
HA7	95	40.5 ± 2.1	36.0 ± 2.8	4.5 ± 0.7	40.0 ± 2.8	4.0 ± 0.0	11.7 ± 0.3	n.d.[c]
	120	208.5 ± 7.8	217.0 ± 9.9	-8.5 ± 2.1	487.5 ± 16.3	270.5 ± 6.4	13.0 ± 0.0	n.d.
	140	230.0 ± 11.3	39.0 ± 14.1	191.0 ± 2.8	298.5 ± 21.9	259.5 ± 7.8	13.0 ± 0.0	n.d.
HA7-WHE	95	440.5 ± 2.1	329.0 ± 1.4	111.5 ± 0.7	518.0 ± 1.4	189.0 ± 0.0	8.9 ± 0.0	93.5 ± 0.6
	120	560.0 ± 12.7	524.5 ± 7.8	35.5 ± 4.9	1173.0 ± 15.6	648.5 ± 7.8	13.0 ± 0.0	92.7 ± 0.0
	140	483.0 ± 11.3	129.0 ± 0.0	354.0 ± 11.3	423.0 ± 12.7	294.0 ± 12.7	13.0 ± 0.0	93.2 ± 0.0
WHE	95	1345.5 ± 2.1	828.5 ± 6.4	341.0 ± 15.6	1920.5 ± 7.8	916.0 ± 5.7	10.1 ± 0.0	89.1 ± 0.0
	120	1213.0 ± 11.3	405.0 ± 4.2	808.0 ± 15.6	1304.0 ± 9.9	899.0 ± 14.1	9.6 ± 0.0	89.3 ± 0.3
	140	1218.0 ± 19.8	160.0 ± 18.4	1058.0 ± 1.4	599.0 ± 17.0	439.0 ± 1.4	9.6 ± 0.0	88.5 ± 0.8
HA7-POT	95	2404.0 ± 9.9	1543.0 ± 12.7	861.0 ± 2.8	1896.0 ± 15.6	353.0 ± 2.8	8.7 ± 0.1	64.8 ± 0.6
	120	2551.5 ± 23.3	967.5 ± 3.5	1584.0 ± 19.8	1661.5 ± 19.1	694.0 ± 15.6	8.9 ± 0.1	65.1 ± 0.0
	140	2550.5 ± 30.4	252.5 ± 2.1	2298.0 ± 28.3	880.0 ± 0.0	627.5 ± 2.1	8.8 ± 0.1	65.1 ± 0.0
POT	95	9081.0 ± 198.0	2088.0 ± 62.2	6920.0 ± 287.1	2722.5 ± 153.4	561.5 ± 64.3	3.8 ± 0.0	61.6 ± 0.0
	120	8684.5 ± 422.1	1004.5 ± 12.0	7680.0 ± 410.1	2088.5 ± 13.4	1084.0 ± 1.4	3.9 ± 0.0	61.6 ± 0.1
	140	8820.0 ± 144.2	429.5 ± 37.5	8390.5 ± 106.8	1475.0 ± 38.2	1045.5 ± 0.7	3.9 ± 0.0	61.6 ± 0.0

[a] Determined using a RVA 4800; starch suspensions of 28.0 g containing 8.0% of dry solids were used for the analysis.
[b] Values are presented as average ± standard deviation (n = 2).
[c] n.d.: not detectable.

3.4.3. Gel strength and microscopic structure of starch gel

After cooking at 95°C and subsequent storage at ambient temperature for 2.0 h, WHE formed the strongest gel among the three starches (gel strength = 75.9 g; **Table 3.4**), whereas HA7 and POT failed to develop a good gel. The discrepancy found in the gelling ability of POT in this and a previous study could be explained by the different sources of the two POT samples (Liu *et al.*, 2019). Under the test conditions, WHE granules swelled to a relatively low extent (**Figure 3.2**) and were able to maintain good integrity after cooking. The swollen WHE granules/remnants with good integrity were able to fill the space in the mold and develop a dense gel network, which was confirmed by the existence of "cells" (indicated by five-point stars) in the lyophilized WHE gel matrix (**Figure 3.3,** WHE – 95°C) (Liu *et al.*, 2019; Ring, 1985). Some amylose molecules (indicated by arrows) appeared between the granules/remnants, which might function as inter-granule/remnant glue to further enhance the gel strength (Daniel, Yao, and Weaver, 2007; Liu *et al.*, 2019). Due to the high T_c (107.2°C; **Table 3.1**), HA7 was only partially gelatinized at 95°C cooking and some granules were able to retain the granular structure (indicated by chevrons; **Figure 3.3,** HA7 – 95°C). Therefore, this high-amylose starch failed to form a strong gel network at 8.0% concentration (w/w, dwb). The repelling between phosphate monoester groups covalently attached to POT amylopectin molecules led to extensive swelling of the granules (**Figure 3.2**). With the continuous heating and stirring during the cooking in RVA, POT lost the granular integrity and was fragmented into small remnants (**Figure 3.3**, POT – 95°C), which was unable to develop a similarly compact gel network filled with cells as noted in WHE gel.

Table 3.4 Gel strength of three native starches and starch blends at 1:1 ratio (dwb) after cooking at 95, 120, and 140°C.[a,b]

Starch	Gel strength (g)		
	95°C	120°C	140°C
HA7	8.5 ± 0.6 a	108.3 ± 3.1 c	604.3 ± 17.6 e
HA7-WHE	34.1 ± 1.9 b (42.2)[c]	82.0 ± 1.1 c (77.2)	109.4 ± 5.9 d (316.6)
WHE	75.9 ± 3.7 c	46.1 ± 4.2 a	28.9 ± 1.4 b
HA7-POT	33.3 ± 0.7 b (8.0)	74.3 ± 3.5 c (56.5)	78.4 ± 3.5 c (304.5)
POT	7.5 ± 0.2 a	4.6 ± 0.1 b	4.7 ± 0.2 a

[a] Starch suspensions (28.0 g containing 8.0% of dry solids) were cooked following the same procedure used for the pasting property determination; the resultant pastes were then poured into a screw-capped plastic canister and stored at ambient temperature for 2.0 h for gelation to take place.

[b] Values are presented as average ± standard deviation (n = 3). The numbers with the same letter in one column were significantly different at $p < 0.05$.

[c] Theoretical gel strength of starch blend = (Gel strength of Starch A × 50%) + (Gel strength of Starch B × 50%).

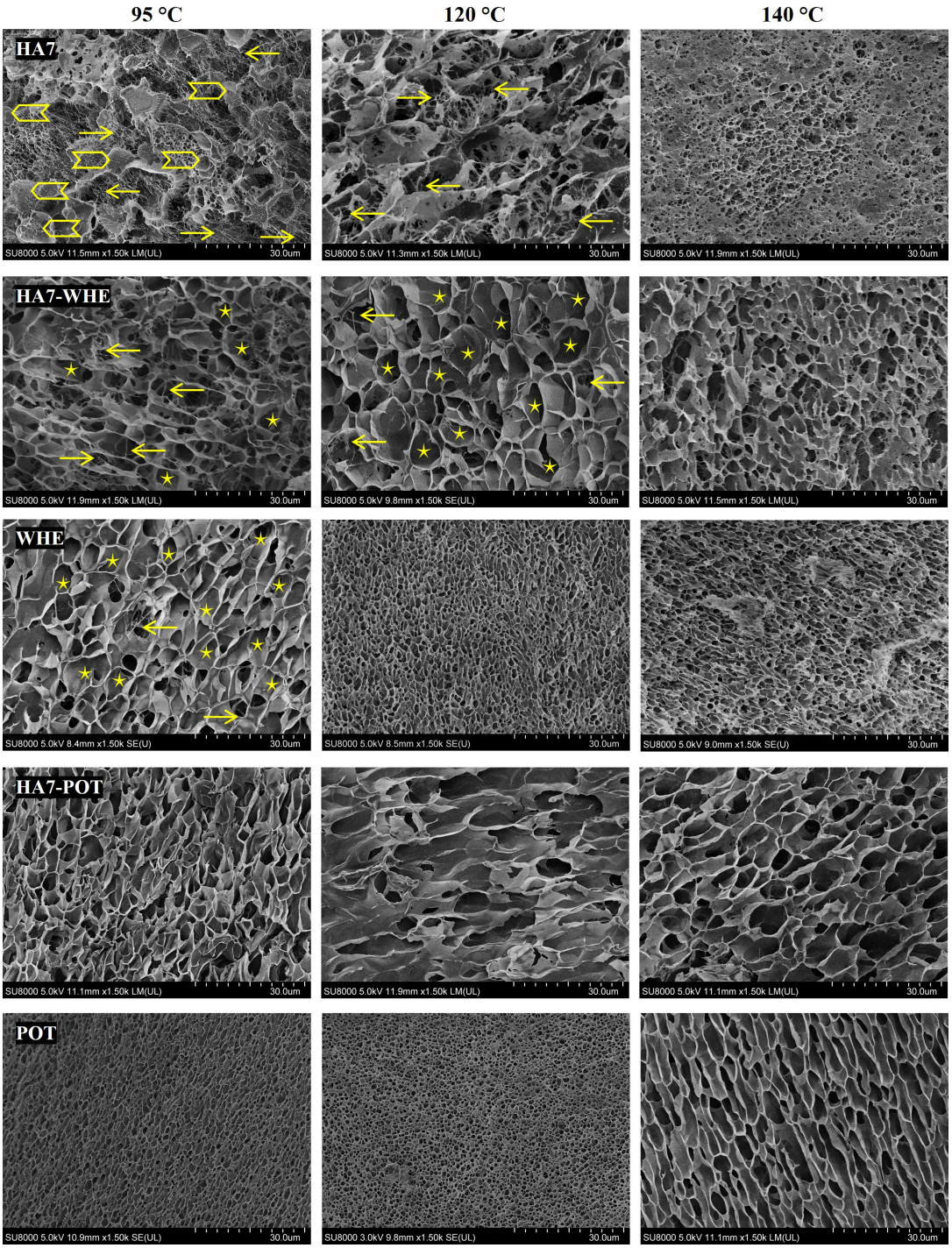


Figure 3.3 Scanning electron microscopy (SEM) images of the cross-sections of the lyophilized starch gels/pastes after cooking three native starches and starch blends at 1:1 ratio (dwb) at 95, 120, and 140°C. After gelation at ambient temperature for 2.0 h , the samples were instantly frozen using liquid nitrogen and then lyophilized prior to viewing under SEM. Five-point stars mark cells in the gel matrix; chevrons mark starch granules partly maintaining the granular morphology after cooking; and arrows mark the amylose existing between starch granules/remnants. All the images were taken at a magnification of 1,500×.

When the holding temperature gradually increased to 140°C, the gel strength of WHE decreased from 75.9 to 28.9 g (**Table 3.4**), which could be explained by the aforementioned thixotropic breakdown and thermal degradation effects (**Section 3.2**) (Liu *et al.*, 2019). The swollen WHE granules/remnants gradually lost the integrity, and the starch molecules became uniformly dispersed to develop a flaky and loose matrix structure at 140°C (**Figure 3.3**, WHE – 140°C), which resembled the matrix structures of POT pastes prepared at 95 and 120°C. Despite the lack of ability to generate a firm gel, the strength of POT paste also decreased at a higher holding temperature. HA7, however, showed the opposite trend in gel strength across the cooking temperature of 95-140°C because this high-amylose starch had a different mechanism to form a strong gel. As discussed in **Section 3.4.2**, a higher heating temperature gelatinized HA7 to a greater extent, which enabled the starch granules to swell and then become well dispersed. The dispersed linear amylose, as the leading constituent in HA7 (63.6%), re-associated with each other to support a compact gel matrix structure (**Figure 3.3,** HA7 – 140°C).

In order to reveal whether there were synergistic or antagonistic effects from the interactions between starch granules/remnants and molecules on the gel formation of starch blends, we also compared the determined gel strength data with the corresponding theoretical values (**Table 3.4**). At 95°C cooking, the measured gel strength of HA7-WHE sample (34.1 g) was slightly lower than the anticipated value (42.2 g), suggesting that the presence of HA7 showed some detrimental effect on the gelling capability of WHE. On the contrary, the determined strength of HA7-POT sample (33.3 g) was more than fourfold of the calculated value (8.0 g), which is in good accordance with the observation that this sample showed somewhat more ordered structure (**Figure 3.3**, HA7-POT – 95°C) than POT alone (**Figure 3.3**, POT – 95°C) after the cooking at 95°C. The result could be explained by that the amylose molecules diffusing out from HA7 were able to limit the extensive swelling of POT granules, which was supported by the observation that the HA7-POT blend only showed 26.5% peak viscosity and a remarkably higher peak time (8.7 *versus* 3.8 min) in comparison with POT alone (**Figure 2** and **Table 3.2** – 95°C). It is interesting to note that the abovementioned HA7 granules partially maintaining the original granular morphology after 95°C cooking were not found in the HA7-WHE nor HA7-POT sample.

As the heating temperature increased from 95 to 120°C, the recorded gel strength of HA7-WHE and HA7-POT samples was consistently higher than the corresponding theoretical values (82.0 *versus* 77.2 g and 74.3 *versus* 56.5 g, respectively; **Table 3**). The data are in good agreement

with the pasting profiles of these two starch blends at 120°C (**Figure 3.2** and **Table 3.3**), where their trough and final viscosities were slightly higher or close to those of WHE and POT, respectively. The higher measured gel strength of HA7-WHE and HA7-POT samples also corresponded well with their more organized matrix structure in comparison with WHE or POT alone (**Figure 3.3** – 120°C). The results suggested that the interactions between starch granules/remnants and molecules could help to maintain the gelling ability of the two starch blends at 120°C cooking.

As the cooking temperature was further increased to 140°C, the determined gel strength of HA7-WHE and HA7-POT samples was noticeably lower than the corresponding calculated values (109.4 *versus* 316.6 g and 78.4 *versus* 304.5 g, respectively; **Table 3.4**). As explained above, the re-association between well-dispersed linear amylose molecules was the key factor for the development of a strong gel from HA7 after cooking at this high temperature. The presence of highly branched amylopectin from the other starch, either WHE or POT, was detrimental for the formation of a dense gel matrix as illustrated in HA7.This explanation was confirmed by the SEM imaging. At 140°C, neither of the starch blends was able to develop a gel matrix as dense as that of HA7 alone (**Figure 3.3**). Instead, their microscopic structures appeared to be similar to that of POT prepared from 140°C cooking, which failed to develop a firm gel (**Table 3.4**). In the complicated systems of starch blends over cooking temperatures of 95-140°C, further research is required to elucidate the interactions between starch granules/remnants and molecules of different botanical origins. The gel strength data presented in our work will be meaningful for other researchers to prepare native starch gels of a broad range of strength under various processing conditions.

3.4.4. *In vitro* digestibility of cooked starch

After cooking in boiling water, HA7 contained 30.5% RS, while those of WHE and POT were substantially lower (~10%; **Table 3.5**). The great enzymatic resistance of HA7 was attributed to the high T_c (107.2°C; **Table 3.1**), and thus a large portion of thermally stable double-helical crystallites remained after 100°C cooking (Jiang *et al.*, 2010). It is worth mentioning that the measured SDS and RS contents of cooked HA7-WHE blend were consistently higher than the anticipated values (4.6% *versus* 2.5% and 23.2% *versus* 20.0%, respectively). The relatively low enzymatic susceptibility of the HA7-WHE sample could be associated with the formation of a

large amount of ALC as shown in **Table 3.1**: (1) ALC is known to be resistant to amylolysis (Jane and Robyt, 1984; Seneviratne and Biliaderis, 1991); (2) The formation of ALC also restricted the swelling of the starch blend upon cooking (**Sections 3.4.2** and **3.4.3**) to further reduce the susceptibility to enzymatic breakdown (Ai *et al.*, 2013; Singh, Chang, Lin, Singh, and Singh, 2011). It is noteworthy that the cooked HA7-POT blend also possessed RS content greater than the theoretical value (23.2% *versus* 20.5%). The observation might be linked to that the leaching of amylose out of HA7 granules into the aqueous medium restricted the swelling and improved the granular integrity of POT (**Figures 3.2** and **3.3,** HA7-POT blend at 95°C), which reduced the accessibility to the amylolytic enzymes. The results from our research suggested that simple blending with HA7 could reduce the enzymatic digestibility of WHE and POT, both of which are common starches found in human diets.

Table 3.5 *In vitro* starch digestibility of three native starches and starch blends after cooking at boiling water temperature [a,b]

Starch	RDS (%)	SDS (%)	RS (%)
HA7	63.0 ± 1.5 a	5.0 ± 0.9 a	30.5 ± 1.0 c
HA7-WHE	69.7 ± 0.3 b	4.6 ± 0.5 a	23.2 ± 0.2 b
	(75.4)[c]	(2.5)	(20.0)
WHE	87.8 ± 1.4 d	0.0 ± 0.0 a	9.5 ± 0.9 a
HA7-POT	70.5 ± 2.6 b	3.9 ± 3.4 a	23.2 ± 0.9 b
	(72.8)	(4.4)	(20.5)
POT	82.6 ± 1.9 c	3.7 ± 2.3 a	10.4 ± 0.4 a

[a] Values are presented as average ± standard deviation (n = 3); the numbers with the same letter in one column are not significantly different at $p < 0.05$.

[b] RDS: rapidly digestible starch, SDS: slowly digestible starch, and RS: resistant starch; values were calculated on a dry weight basis.

[c] Theoretical %RDS/SDS/RS of cooked starch blend = (%RDS/SDS/RS of cooked Starch A × 50%) + (%RDS/SDS/RS of cooked Starch B × 50%).

3.5. Conclusions

In the current study, we clearly demonstrated that blending WHE or POT with high-amylose HA7 at 1:1 ratio (dwb) could be a "clean-label" and effective approach to generate starch ingredients with diversified functionality and reduced *in vitro* digestibility. In an excess amount of water (starch:water = 1:3), the gelatinization behaviors of the individual starches appeared to be independent during the DSC scanning of HA7-WHE and HA7-POT blends. However, there was a higher amount of single-helical ALC developed in the HA7-WHE blend, which could partially contribute to to to the markedly small percentage of retrogradation (9.2%) of the gelatinized sample after the 7-day storage at 4°C. Different from HA7-POT blend, HA7-WHE blend showed remarkably higher peak, trough, and final viscosities at 120°C heating than 95 and 140°C, suggesting the marked resistance of HA7-WHE blend against thixotropic breakdown and thermal degradation at high-temperature cooking of 120°C. Both HA7-WHE and HA7-POT blends were able to form starch gels of moderate strength (74.3-109.4 g) after 120 and 140°C cooking. Additionally, the water-boiled HA7-WHE sample exhibited lower RDS content but higher SDS and RS contents than the data calculated by simply assuming additivity, indicating the enhanced enzymatic resistance of the blend, which could be associated with the formation of a high amount of ALC. In summary, the obtained HA7-WHE blend possessed the following attractive techno-functional attributes: (1) Having a low retrogradation rate after gelatinization; (2) Displaying moderate thickening and gelling ability at 120°C cooking; and (3) Being a good source of SDS (4.6%) and RS (23.2%) after being boiled in water. This starch blend will be suitable for a broad range of food applications, particularly for canning, retorting, and jet-cooking that typically have a high processing temperature close to 120°C.

3.6 Connection to next study

Extrusion is a continuous, short-time process that can transform raw ingredients, such as starches and flours, into desired products through mixing, kneading, shearing, cooking, melting, structuring, and/or shaping. It has been widely used in the food industry to modify ingredients and manufacture final products due to its high versatility, efficiency, and relatively low operating cost. Starch modified by extrusion possesses unique functionalities, such as high water-holding capacity, great water solubility, and instant viscosity. Recently, extrusion processing of high-amylose maize starch (HAMS) has attracted research attention because a large portion of resistant

starch (RS) can be retained in the extrudate (Shrestha *et al.*, 2010; Zhang *et al.*, 2015). In the current literature, there are few studies investigating the impacts of extrusion modification on the functionality and digestibility of starch blends. Because of the attractive attributes of HA7-WHE and HA7-POT starch blends as identified in Study 1, in the subsequent study we aimed to examine: (1) The effects of extrusion modification on the functional profiles and *in vitro* digestibility of the starch blends; (2) How the modification effects are determined by the applied extrusion conditions.

4 PASTING AND GELLING PROPERTIES AND *IN VITRO* DIGESTIBILITY OF STARCH-BLEND EXTRUDATES

4.1 Abstract

Extrusion has been widely used in the food industry to modify ingredients and manufacture final products due to its high versatility, efficiency, and relatively low operating cost. However, limited information is available on the impact of extrusion on the functionality and digestibility of starch blends in the literature. This research aimed to examine how extrusion affected the functional profiles and *in vitro* digestibility of starch blends. High-amylose maize starch (HA7) was blended with wheat (WHE) or potato starch (POT) at 1:1 ratio (dry weight basis; dwb), and the two starch blends and three individual starches were extruded under two sets of extrusion conditions: "mild" (high feed moisture, low temperature, and low screw speed) and "extreme" (low feed moisture, high temperature, and high screw speed). As expected, the extreme condition generally led to a higher specific mechanical energy (SME) on the same starch sample, which was responsible for higher degrees of starch gelatinization and degradation, particularly for POT and HA7-POT blend. SME, amylose contents, and damaged/gelatinized starch contents of the starch blend extrudates were in between those of the corresponding extruded individual starches. Greater levels of starch gelatinization, granular disintegration, and molecular degradation caused by extrusion led to higher cold paste viscosities and lower overall pasting viscosities of the starch extrudates. Largely consistent with the pasting profiles, the changes mentioned above induced by extrusion diminished the ability of the starch blends and individual starches to form strong gels after cooking and storage. Additionally, in general, HA7-WHE and HA7-POT extrudates showed similar in vitro digestibility compared with their counterparts boiled in water, suggesting that the extrusion of starch blends did not further promote molecular interactions to enhance the enzymatic resistance. The information obtained from the study will provide new directions for the food industry to produce pre-gelatinized starch ingredients with enhanced functional and nutritional properties.

4.2 Introduction

Extrusion cooking is a continuous processing technology that has been widely employed by food industry to produce food ingredients and products. It is a combination of multiple unit operations: mixing, cooking, kneading, shearing, and/or forming. With the applied high shear and temperature and the high pressure inside the extruder barrel, the food ingredients will undergo a series of physicochemical transformations during the processing (*e.g.,* gelatinization of starch, denaturation of protein, formation of amylose-lipid complexes). Starch, in particular, experiences loss of granular integrity and crystalline structure and molecular breakdown, which consequently results in drastic changes in its functional and nutritional properties (Lai and Kokini, 1991; Ye *et al.*, 2018). The high temperature and shear inside extruder barrel gelatinize and dextrinize starch, which thus increases the water absorption index, water solubility index, instant viscosity, and fluidity of starch (Ye *et al.*, 2018). In recent years, researchers are striving for producing starch-based food ingredients with enhanced RS content (Cervantes-Ramírez *et al.*, 2020; Hasjim and Jane, 2009; Masatcioglu, Sumer, and Koksel, 2017; Shrestha *et al.*, 2010), and high-amylose maize starch attracts much attention due to its inherent high RS content (Li *et al.*, 2008).

In **Chapter 3**, we have demonstrated that blending high-amylose maize starch (HA7) with wheat (WHE) or potato starch (POT) can be a "clean-label" approach to generate starch ingredients with diversified functionality and *in vitro* digestibility profiles. Specifically, HA7-WHE blend at 1:1 ratio (dwb) showed markedly reduced tendency to retrograde and exceptional resistance to thixotropic breakdown and thermal degradation during high-temperature cooking at 120°C. In the current study, our main objective was to examine whether extrusion could further enhance the functional properties and diversify the *in vitro* digestibility profiles of the same HA7-WHE and HA7-POT starch blends, and how the used extrusion conditions determined such effects. The two starch blends were extruded with two sets of conditions, and the thermal properties, pasting and gelling attributes over heating temperatures of 95–140°C, and *in vitro* digestibility of resultant starch extrudates were determined and compared with those of their native counterparts. The obtained information will provide new directions for the food industry to produce starch ingredients with enhanced functional and nutritional properties.

4.3 Materials and Methods

4.3.1 Materials

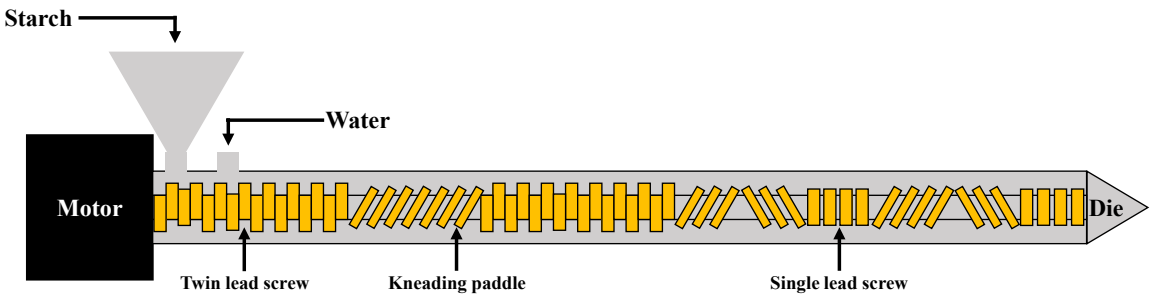
High-amylose maize (HA7), wheat (WHE), and potato (POT) starches and their blends (*i.e.,* HA7-WHE and HA7-POT at 1:1 ratio, dry weight basis) were prepared according to **Section 3.3.1**. Two batches of each blend were prepared. Besides the chemicals and enzymes mentioned in **Section 3.3.1**, potato amylose and Starch Damage Assay Kit were purchased from Megazyme Ltd. (Co. Wicklow, Ireland). Maize amylopectin was procured from Sigma-Aldrich Canada Co. (Oakville, ON, Canada).

4.3.2 Extrusion of starch

The three native starches and the starch blends were extruded using a co-rotating twin-screw extruder (**Figure 4.1**; Model MP19T2-25, APV Baker Inc., Grand Rapids, MI, U.S.A.) with a 19 mm barrel diameter, an L/D ratio of 25:1, and a 6 mm diameter exit die (Kim, Tanhehco, and Ng, 2006). The two sets of extrusion conditions, namely “mild” and “extreme”, are summarized in **Figure 4.1**. The raw starch sample was fed into the extruder using a volumetric feeder (Model K2V-T20, Coperion K-Tron Pitman Inc., Sewell, NJ, U.S.A.) at 1.8 kg/h, and the total feed moisture (*i.e.*, 40% for mild and 25% for extreme condition) was adjusted using a water pump (Brook Crompton E2 Metripump, Hudders Field, U.K.). The specific mechanical energy (SME) during extrusion was calculated according to the following equation:

SME (Wh/kg) = [(Test screw speed / Rated screw speed) × (%Motor load × Rated motor power) / Feed rate] / 100,

where the rated screw speed and the rated motor power input for the extruder were 500 rpm and 2.0 kW, respectively, and the feed rate (kg/h) was the total mass input of feed and injected water.



	Screw speed (rpm)	Moisture (%)	Zone I (°C)	Zone II (°C)	Zone III (°C)	Zone IV (°C)	Zone V (°C)
Mild	100	40	50	50	70	90	110
Extreme	200	25	60	80	100	120	140

Figure 4.1 Schematic illustration and screw configuration of the co-rotating twin-screw extruder used in this study

Each starch sample was extruded under "mild" or "extreme" condition in two independent batches. The collected extrudates were dried at 40°C using a commercial dehydrator overnight (COMM2, Excalibur, Sacramento, CA, U.S.A.), followed by milling using a hammer mill (Lab Mill 3100, Perten Instrument, Winnipeg, MB, Canada) to pass through a 0.8-mm sieve to prepare fine starch powders for the subsequent analyses.

4.3.4. Amylose content of native starch and starch extrudate

Amylose contents of the milled individual starch and starch-blend extrudates as well as their respective native counterparts were determined using an iodine colorimetric method of Chrastil (1987). In brief, 100.0 mg of starch sample was dispersed in 6.0 mL of 90% (v/v) dimethyl sulfoxide (DMSO) by heating in a boiling water bath with gentle magnetic stirring. After cooling to room temperature, a 2.0-mL aliquot of the dispersion was mixed with 10.0 mL of anhydrous ethanol and centrifuged at 3,000 *g* for 15 min. The supernatant was carefully decanted, and the defatted starch pellet was re-dispersed in 6.0 mL of urea-DMSO (0.6 M urea in 90% DMSO) by heating in a boiling water bath with mild magnetic stirring for 30 min. After cooling to ambient temperature, a 100-μL aliquot of the starch dispersion was mixed with 5.0 mL of 0.5% (v/v) trichloroacetic acid and 50 μL of 0.01 M iodine-potassium iodide (I_2-KI) solution to develop color. After being kept at room temperature for 30 min, the absorbance of the mixture was measured at 620 nm against water blank using a spectrophotometer. A standard curve was constructed using

the blends of commercial potato amylose and maize amylopectin standards to give 0.0%, 25.0%, 50.0%, 75.0% and 100.0% amylose.

4.3.5. Content of damaged/gelatinized starch of starch extrudate

Contents of damaged/gelatinized starch of the milled individual starch and starch-blend extrudates were determined in accordance with AACC Method 76-31.01 using Megazyme Starch Damage Assay Kit (AACC Approved Methods of Analysis, 1999; Liu, Yin, Pickard, and Ai, 2020).

4.3.3. Pasting and gelling properties of starch extrudate

Pasting and gelling properties of the milled individual starch and starch-blend extrudates were determined according to **Section 3.3.3**. Briefly, the ground starch extrudate slurry (28.0 g total weight, 8.0% dry solids) was pasted in a Rapid Visco Analyzer (RVA 4800, Perten Instruments Australia Pty Ltd., Sydney, Australia) using the following temperature profile: 1) equilibrated at 50°C for 1 min; 2) heated to the target holding temperature (*i.e.* 95, 120, or 140°C) at a rate of 6°C/min; 3) kept at the holding temperature for 5 min; 4) cooled to 50°C at a rate of 6°C/min; 5) kept at 50°C for 2 min. Pasting temperature, peak viscosity, trough viscosity, breakdown viscosity, final viscosity, setback viscosity, and peak time of the milled starch extrudate were analyzed using TCW Software (Version 3.17.2.487; Perten Instruments Australia Pty Ltd.).

After the cooking at 95, 120, or 140°C in RVA 4800, the obtained starch extrudate paste was transferred into a screw-capped plastic canister (internal diameter = 33.0 mm) to achieve a height of approximately 25 mm. The container was capped and then kept at ambient temperature (~22°C) for 2.0 h for the paste to gel. The texture of the extrudate gel was analyzed using a texture analyzer (TA.XT. Plus, Texture Technologies Corp., South Hamilton, MA, U.S.A.) equipped with a TA-10 cylindrical probe (diameter = 12.7 mm; Texture Technologies Corp.). When a trigger force of 0.5 g was reached, the probe proceeded to penetrate the gel for 10.0 mm at a speed of 0.5 mm/s. The maximum positive force determined during the compression was recorded as the gel strength. Theoretical gel strength of the starch-blend extrudates was calculated using the following equation:

Theoretical gel strength of starch-blend extrudate = (Gel strength of Starch A extrudate × 50%) + (Gel strength of Starch B extrudate × 50%).

4.3.6 *In vitro* digestibility of starch extrudate

In vitro digestibility of the milled individual starch and starch-blend extrudates was determined according to **Section 3.3.6**. The ground starch extrudate containing 600 mg of starch was suspended in 15.0 mL of distilled water, followed by the addition of 5.0 mL of sodium acetate buffer (pH 5.2, 0.4 M, containing 0.18% calcium chloride and 0.08% sodium azide, w/v). Then, the sample was equilibrated in a 37°C shaking water (160 rpm) for approximately 15 min, and 5.0 mL of enzyme cocktail containing porcine pancreatin extract and amyloglucosidase was pipetted into the test tube to start the enzymatic hydrolysis. The amounts of glucose released from the amylolysis at 20 and 120 min were analyzed using Megazyme D-glucose Assay Kit. The contents of rapidly digestible starch (RDS; digested within 20 min), slowly digestible starch (SDS; digested between 20 and 120 min), and resistant starch (RS; undigested after 120 min) were calculated according to the equations described by Englyst *et al.* (1992). Theoretical RDS/SDS/RS contents of the starch-blend extrudates were calculated using the following equation:

Theoretical %RDS/SDS/RS of starch-blend extrudate = (%RDS/SDS/RS of Starch A extrudate × 50%) + (%RDS/SDS/RS of Starch B extrudate × 50%).

4.3.7 Statistical analysis

Each of the individual starch and starch-blend samples was extruded under “mild” or “extreme” condition in two independent batches, and each batch of the extrudates was analyzed in duplicate for all the experiments (n = 4). Statistical differences among the individual starch and starch-blend extrudates were evaluated using one-way ANOVA, Tukey's multiple comparison test at a significance level of 0.05 in IBM SPSS Statistics Analysis (Version 27.0).

4.4 Results and Discussion

4.4.1 Extrusion of starch

Larger SME input was observed for extruding the individual starch and starch-blend samples under the extreme condition (**Table 4.1**) due to the higher screw speed and lower feed moisture (**Figure 4.1**), which was consistent with previous studies using a twin-screw extruder (Ai, Cichy, Harte, Kelly, and Ng, 2016; Köksel, Ryu, Basman, Demiralp, and Ng, 2004). According to the equation shown in **Section 4.3.2**, SME was proportional to the applied screw

speed and the recorded motor load; and a lower moisture level could lead to a higher viscosity of the material in the barrel as well as a subsequent greater motor load. Die pressure largely reflects the constrained flow of material through the die opening, and thus a higher screw speed and a lower feed moisture level in the extreme condition increased the die pressure as shown in **Table 4.1**.

HA7 generally demonstrated the highest percentage of motor load and die pressure under the same condition, followed by POT and WHE. The largest percentage of motor load and die pressure of HA7 could be ascribed to the exceptionally high gelatinization temperatures (T_c = 107.2°C; **Section 3.4.1**), which could possess the highest resistance against extrusion process and thus display the largest percentage of motor load and die pressure. The presence of phosphate monoesters in POT resulted in remarkably high viscosity development upon gelatinization (Shi *et al.*, 2002), which could be partially responsible for the greater percentage of motor load and die pressure during the extrusion of POT in comparison with the processing of WHE. As illustrated in **Section 3.4.2**, WHE exhibited the lowest pasting viscosity among all the three native starches due to the fact that the formation of amylose-lipid complexes (ALC) restricted granular swelling of this starch.

For extruding HA7-WHE blend, the percentage of motor load, die pressure, and SME were in between the values of processing HA7 or WHE alone or close to the values under both mild and extreme conditions. However, the percentage of motor load, die pressure, and SME of extruding HA7-POT blend were in general lower than the values of processing HA7 or POT under both conditions, suggesting that this starch blend could be more easily extruded.

Table 4.1 Extrusion conditions used to extrude individual starches and starch blends.[a]

	Mild					Extreme				
	Feeding rate (kg/h)	Moisture (%)	Motor load (%)	Die pressure (psi)	SME (Wh/kg)	Feeding rate (kg/h)	Moisture (%)	Motor load (%)	Die pressure (psi)	SME (Wh/kg)
HA7	1.8	40	25.8 ± 0.4 d	75.0 ± 7.1 b	42.1 ± 0.6 d	1.8	25	31.3 ± 0.3 b	85.0 ± 0.0 c	58.9 ± 0.5 bc
HA7:WHE	1.8	40	21.6 ± 0.0 b	30.0 ± 0.0 a	34.7 ± 0.1 b	1.8	25	31.5 ± 0.4 b	47.5 ± 10.6 ab	58.3 ± 0.7 b
WHE	1.8	40	19.4 ± 0.2 a	30.0 ± 0.0 a	30.5 ± 0.4 a	1.8	25	27.3 ± 0.8 a	25.0 ± 7.1 a	49.6 ± 1.5 a
HA7:POT	1.8	40	22.7 ± 0.2 bc	27.5 ± 3.5 a	38.6 ± 0.4 c	1.8	25	30.5 ± 0.1 b	55.0 ± 0.0 b	59.8 ± 0.2 bc
POT	1.8	40	23.4 ± 0.7 c	35.0 ± 0.0 a	41.3 ± 1.2 cd	1.8	25	31.5 ± 1.3 b	65.0 ± 7.1 bc	64.2 ± 2.6 c

[a]Measured values are presented as the average ± standard deviation (n = 2); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.
[b]SME = [(Test screw speed / Rated screw speed) × (%Motor load × Rated motor power) / Feed rate] / 100, where the rated screw speed and the rated motor power input for the extruder were 500 rpm and 2.0 kW, respectively; and the feed rate (kg/h) was the total mass input of feed and injected water.

4.4.2 Amylose content of native starch and starch extrudate

Amylose contents of HA7-WHE and HA7-POT samples generally reflected the features of the individual starches (**Table 4.2**). Therefore, the native starch samples showed amylose content in an ascending order of POT < WHE < HA7-POT < HA7-WHE < HA7. The mild and extreme extrusion conditions reduced the amylose contents of HA7, HA7-WHE, and WHE from 63.6%, 52.7%, and 35.2% to ~56%, ~43%, and ~33%, respectively, suggesting that extrusion effectively degraded amylose (most likely amylopectin too). However, the two processing conditions did not display different effects on the amylose contents of the three starch samples. The mild and extreme conditions diminished the amylose content of POT from 24.8% to 16.9% and 7.7%, respectively, suggesting that the extreme condition degraded amylose (most likely amylopectin too) of this starch to a greater extent. The observation could be related to that POT was able to swell to a higher level than HA7 and WHE after gelatinization (**Section 3.4.2**), and thus the molecules in gelatinized POT become more susceptible to degradation, particularly in the extreme processing. For the same reason, the mild condition reduced the amylose content of HA7-POT from 47.4% to 44.3%, which was further decreased to 39.8% by the extreme condition.

Table 4.2 Amylose contents of native individual starches and starch blends and their extrudates from mild and extreme conditions.[a]

	Amylose (%)		
	Native	Extruded	
		Mild	Extreme
HA7	63.6 ± 0.9 e	55.8 ± 1.3 d	55.6 ± 0.6 e
HA7-WHE	52.7 ± 0.7 d	42.3 ± 0.4 c	43.5 ± 0.5 d
WHE	35.2 ± 0.8 b	33.4 ± 0.6 b	32.0 ± 0.9 b
HA7-POT	47.4 ± 0.6 c	44.3 ± 0.8 c	39.8 ± 2.8 c
POT	24.8 ± 2.6 a	16.9 ± 1.5 a	7.7 ± 1.8 a

[a] Values are presented as average ± standard deviation (n = 4); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

4.4.3 Content of damaged/gelatinized starch of starch extrudate

Among HA7, WHE, and POT, HA7 extrudates showed significantly lower damaged/gelatinized starch contents than WHE and POT extrudates under both extrusion conditions (**Table 4.3**), which could be attributed to the considerably higher gelatinization temperatures of HA7 (**Section 3.4.1**). As expected, the contents of damaged/gelatinized starch of

HA7-WHE and HA7-POT extrudates were in between those of their individual starch extrudates. All the extrudates obtained from the extreme condition, namely higher temperature, shear, die pressure, and SEM, possessed higher contents of damaged/gelatinized starch than the counterparts from the mild condition, which is consistent with the data reported in the literature (Cai, Diosady, and Rubin, 1995).

Table 4.3 Contents of damaged/gelatinized starch of individual starch and starch-blend extrudates from mild and extreme conditions.[a]

	Damaged/gelatinized starch (%)	
	Mild	Extreme
HA7	12.7 ± 0.9 a	20.4 ± 0.6 a
HA7-WHE	43.8 ± 0.7 c	47.9 ± 2.4 b
WHE	54.3 ± 4.7 d	69.1 ± 1.8 d
HA7-POT	28.7 ± 1.0 b	58.1 ± 0.5 c
POT	48.5 ± 1.1 c	85.2 ± 0.4 e

[a] Values are presented as average ± standard deviation (n = 4); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

4.4.4 Pasting properties of starch extrudate

Pasting properties of the individual starch and starch-blend extrudates from mild and extreme conditions determined at heating temperatures of 95, 120, and 140°C are presented in **Figure 4.2** and **Table 4.4**. Compared with their respective native counterparts (**Section 3.4.2**), overall the extrusion treatments led to the following changes in the pasting properties of all the five starch samples: (1) The extrudates were able to develop noticeable pasting viscosities at a lower temperature (except for HA7), which could be associated with their remarkably high contents of damaged/gelatinized starch to provide instant viscosity (**Table 4.4**) (Bhattacharya, Sudha, and Rahim, 1999; Gomez and Aguilera, 1983). (2) The extrudates exhibited considerably lower pasting properties, which could be attributed to starch gelatinization (**Table 4.4**), loss of granular structure, and molecular degradation resulting from extrusion (**Table 4.2** and **4.3**). More importantly, the described impacts were more obvious with the extreme condition. Overall, the pasting viscosities of the starch extrudates were in between those of their corresponding individual starch extrudates (**Figure 4.2** and **Table 4.4**). Notably, the POT extrudate from the extreme condition had instant viscosity at 50°C, but the pasting viscosity was low, even lower than that of HA7-POT. The noted low pasting viscosity of POT extrudate from the extreme condition corresponds well with the greatest extent of molecular degradation (**Table 4.2**) and starch gelatinization (**Table 4.4**).

After being processed under the mild condition, As the holding temperature increased from 95°C to 120 and 140°C in RVA 4800, the peak viscosity of POT extrudate from the mild condition remained the same, while that of WHE slightly decreased, similar to the changes observed in their native counterparts (**Section 3.4.2**). Relatively higher trough and final viscosities were observed for HA7-WHE blend extrudate pasted at 120°C among three applied holding temperatures. However, the same trend was not observed with HA7-POT extrudate. The results suggested that the interaction as described in **Section 3.4.2** could be retained after the extrusion under the mild condition. Interestingly, the aforementioned features of HA7-WHE blend extrudate were not observed in the extreme counterpart.

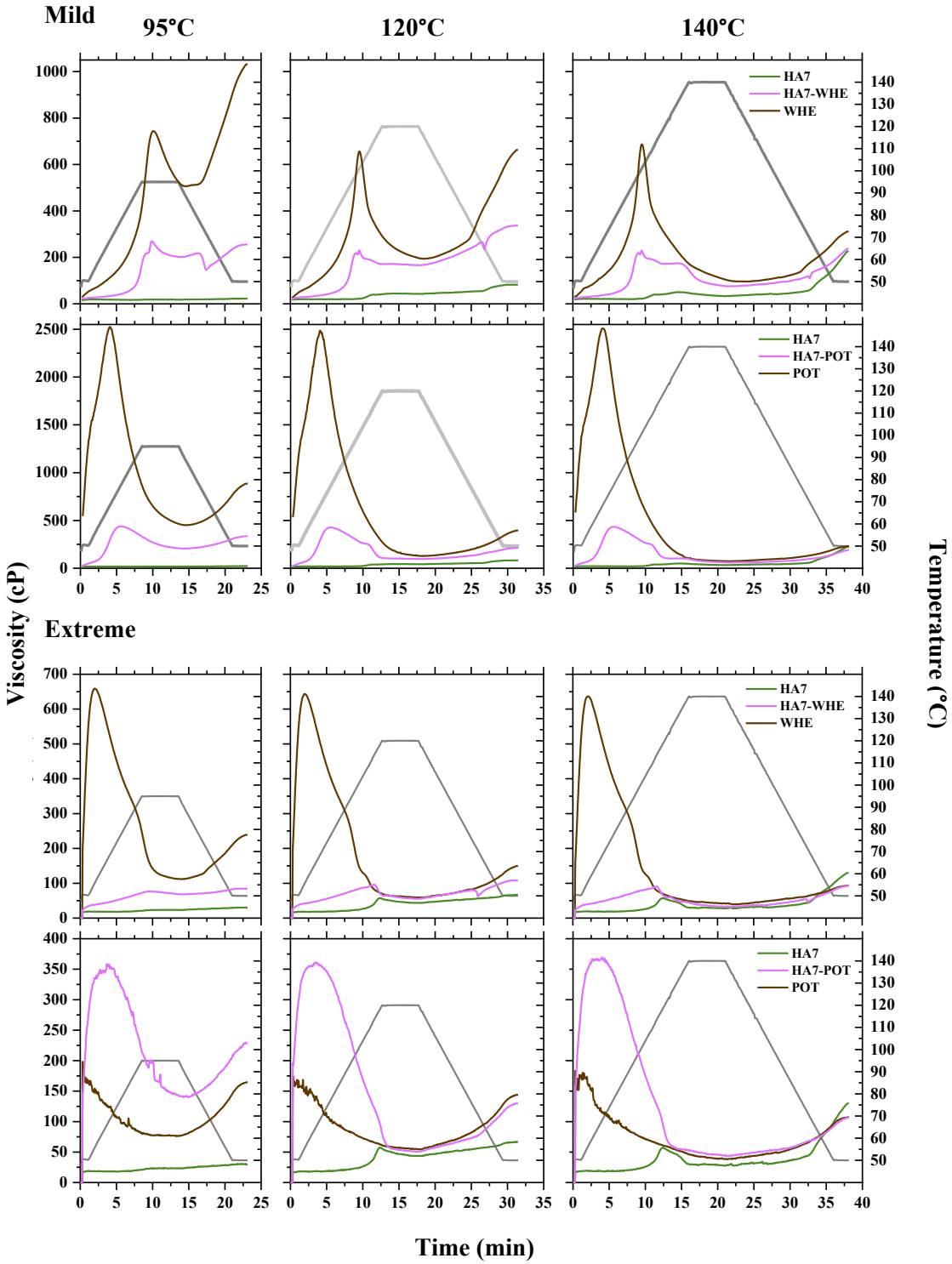


Figure 4.2 Pasting profiles of individual starch and starch-blend extrudates from mild and extreme conditions analyzed at holding temperatures of 95, 120, and 140°C. Starch suspensions (28.0 g in total) containing 8.0% of dry solids were used for the analysis.

Table 4.4 Pasting parameters of individual starch and starch-blend extrudates from mild and extreme conditions analyzed at holding temperature of 95, 120, and 140°C.[a,b]

	Holding temperature (°C)	Pasting viscosity (cP)					Peak time (min)
		Peak	Trough	Breakdown	Final	Setback	
Mild							
HA7	95	19.5 ± 1.3	17.3 ± 1.9	2.3 ± 1.0	22.5 ± 2.4	5.3 ± 0.5	4.7 ± 3.0
	120	42.0 ± 2.6	43.0 ± 1.4	-1.0 ± 1.4	82.5 ± 4.1	39.5 ± 3.1	12.9 ± 0.1
	140	42.8 ± 3.3	33.3 ± 3.9	9.5 ± 3.7	226.0 ± 37.2	192.8 ± 39.2	12.9 ± 0.1
HA7-WHE	95	270.3 ± 3.9	145.3 ± 6.9	125.0 ± 10.0	255.8 ± 8.4	110.5 ± 3.7	9.9 ± 0.1
	120	230.8 ± 3.6	165.3 ± 2.2	65.5 ± 1.7	336.8 ± 3.3	171.5 ± 3.1	9.5 ± 0.0
	140	229.5 ± 2.1	76.5 ± 2.4	153.0 ± 1.4	237.3 ± 10.3	160.8 ± 10.6	9.5 ± 0.0
WHE	95	745.0 ± 15.7	506.8 ± 28.3	238.3 ± 13.6	1031.5 ± 27.3	524.8 ± 7.2	10.1 ± 0.1
	120	657.3 ± 27.1	193.5 ± 3.3	463.8 ± 27.1	662.5 ± 8.7	469.0 ± 9.0	9.5 ± 0.1
	140	687.8 ± 20.4	105.3 ± 10.1	582.5 ± 10.3	311.5 ± 5.5	206.3 ± 13.3	9.5 ± 0.0
HA7-POT	95	438.5 ± 24.3	206.3 ± 8.0	232.3 ± 30.4	335.8 ± 9.6	129.5 ± 2.4	5.5 ± 0.1
	120	428.0 ± 17.6	96.5 ± 4.2	331.5 ± 21.7	213.0 ± 11.2	116.5 ± 8.2	5.5 ± 0.0
	140	433.8 ± 27.7	61.0 ± 7.1	372.8 ± 33.4	190.8 ± 10.3	129.8 ± 4.4	5.5 ± 0.1
POT	95	2531.8 ± 44.7	450.8 ± 10.4	2081.0 ± 34.7	885.3 ± 25.3	434.5 ± 15.2	4.1 ± 0.1
	120	2480.3 ± 215.7	128.3 ± 9.5	2352.0 ± 219.9	395.5 ± 21.2	267.3 ± 12.5	4.1 ± 0.1
	140	2517.5 ± 71.0	74.5 ± 9.5	2443.0 ± 71.7	229.8 ± 2.9	155.3 ± 7.1	4.1 ± 0.2
Extreme							
HA7	95	23.8 ± 2.4	22.5 ± 2.4	1.3 ± 0.5	29.8 ± 2.9	7.3 ± 0.5	9.9 ± 0.5
	120	57.5 ± 2.4	43.3 ± 2.8	14.3 ± 1.0	66.8 ± 3.8	23.5 ± 1.3	12.3 ± 0.1
	140	55.0 ± 6.8	24.3 ± 7.7	30.8 ± 1.0	126.5 ± 9.1	102.3 ± 2.6	12.4 ± 0.0
HA7-WHE	95	76.8 ± 4.6	67.3 ± 4.6	9.5 ± 0.6	84.5 ± 5.4	17.3 ± 1.0	9.3 ± 0.2
	120	96.3 ± 4.4	54.8 ± 3.8	41.5 ± 1.0	107.8 ± 8.0	53.0 ± 4.2	11.5 ± 0.1
	140	91.0 ± 3.6	32.5 ± 1.9	58.5 ± 3.7	93.0 ± 1.8	60.5 ± 2.1	11.5 ± 0.1
WHE	95	602.8 ± 22.4	111.5 ± 3.5	491.3 ± 18.9	239.0 ± 7.1	127.5 ± 3.7	3.1 ± 0.0
	120	583.3 ± 26.4	58.5 ± 4.8	524.8 ± 21.8	149.5 ± 12.7	91.0 ± 8.3	3.1 ± 0.0
	140	583.5 ± 27.1	41.3 ± 2.5	542.3 ± 28.2	93.5 ± 2.5	52.3 ± 3.2	3.1 ± 0.0
HA7-POT	95	358.8 ± 32.2	138.3 ± 7.9	220.5 ± 26.3	228.5 ± 11.3	90.3 ± 9.7	3.9 ± 0.9
	120	362.0 ± 9.6	50.8 ± 2.6	311.3 ± 11.8	129.8 ± 5.1	79.0 ± 3.2	3.6 ± 0.3
	140	376.3 ± 5.3	44.3 ± 10.2	332.0 ± 14.4	106.3 ± 9.3	62.0 ± 1.8	3.7 ± 0.6
POT	95	131.8 ± 13.8	76.3 ± 10.1	55.5 ± 8.5	164.5 ± 16.7	88.3 ± 6.8	3.2 ± 0.2
	120	147.8 ± 4.9	54.0 ± 1.8	93.8 ± 6.2	143.8 ± 2.1	89.8 ± 1.5	3.2 ± 0.3
	140	128.3 ± 6.8	38.3 ± 3.0	90.0 ± 6.6	107.3 ± 4.3	69.0 ± 1.4	3.4 ± 0.3

[a] Determined using a RVA 4800; starch extrudate suspensions of 28.0 g containing 8.0% of dry solids were used for the analysis.
[b] Values are presented as average ± standard deviation (n = 4).
[c] n.d.: Not detectable.

4.4.5 Gel strength of starch extrudate

Gel strength of the individual starch and starch-blend extrudates after cooking at 95, 120, and 140°C is shown in **Table 4.5**. In general, the gelatinization and molecular degradation induced by extrusion as described above disintegrated the starch granules and decreased their ability to form a compact gel network. Thus, the gel strength values of the starch extrudates was considerably lower compared with those of their native counterparts (**Section 3.4.3**), and the starch extrudates from the extreme condition possessed lower gel strength readings compared with the counterparts from the extreme condition. Meanwhile, similar to those of their native counterparts (**Section 3.4.3**), gel strength values of WHE and POT extrudates gradually decreased as the holding temperature increased from 95 to 140°C, which was attributed to the molecular degradation and thixotropic break down during pasting as discussed previously. The opposite trend was observed for HA7 extrudates as the gel strength increased when the holding temperature was elevated, which could be explained by that the heating temperature of 140°C was able to fully gelatinize HA7 extrudates to allow the linear amylose molecules to re-associate to form a compact gel matrix (**Section 3.4.3**).

After cooking at 95 and 120°C, HA7-WHE extrudate from the mild condition showed gel strength values slightly higher than the corresponding theoretical data (**Table 4.5**). However, for the HA7-WHE extrudate from the mild condition and the HA7-POT from both conditions, the determined gel strength data were consistently smaller than the calculated values at the three cooking temperatures, suggesting that the extrusion processing lowered the gelling ability of the two starch blends.

Table 4.5 Gel strength of individual starch and starch-blend extrudates from mild and extreme conditions after cooking at 95, 120, and 140°C.[a,b]

	Gel strength (g)					
	Mild			Extreme		
	95°C	120°C	140°C	95°C	120°C	140°C
HA7	3.9 ± 0.8 a	12.2 ± 1.3 a	368.4 ± 29.2 e	9.9 ± 3.7 b	36.7 ± 7.6 c	295.6 ± 22.2 c
HA7-WHE	32.9 ± 2.7 c (27.8)[c]	30.3 ± 2.9 c (17.1)	94.1 ± 3.3 d (194.6)	3.9 ± 0.1 b (13.3)	15.7 ± 1.0 b (21.3)	50.1 ± 3.2 b (150.2)
WHE	51.6 ± 8.7 d	22.0 ± 0.8 b	20.7 ± 0.4 b	16.7 ± 0.9 c	5.8 ± 0.2 a	4.8 ± 0.4 a
HA7-POT	4.8 ± 0.5 a (10.9)	12.5 ± 1.0 a (13.7)	53.9 ± 8.7 c (189.6)	3.5 ± 0.3 b (6.3)	13.8 ± 2.8 b (19.6)	46.1 ± 6.8 b (149.1)
POT	17.9 ± 1.5 b	15.1 ± 0.5 a	10.7 ± 0.9 a	2.7 ± 0.1 a	2.5 ± 0.1 a	2.6 ± 0.0 a

[a] Starch extrudate suspensions (28.0 g containing 8.0% of dry solids) were cooked following the same procedure used for the pasting property determination; the resultant pastes were poured into a plastic canister and stored at ambient temperature for 2.0 h for gelation.
[b] Values are presented as average ± standard deviation (n = 4); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.
[c] Theoretical gel strength of starch-blend extrudate = (Gel strength of Starch A extrudate × 50%) + (Gel strength of Starch B extrudate × 50%).

4.4.6 *In vitro* digestibility of starch extrudate

The *in vitro* digestibility of the individual starch and starch-blend extrudates is summarized in **Table 4.6**. HA7 extrudate from the mild condition possessed considerably lower RDS (54.9% *versus* 63.6%), higher SDS (12.9% *versus* 5.0%), and slightly lower RS (27.4% *versus* 30.5%) content in comparison with the native counterpart after water boiling, suggesting that HA7 extrudate was less susceptible to amylolysis than the water-boiled HA7. The observation is in agreement with the data reported in a previous study (Htoon *et al.*, 2009), which could be attributed to that the extrusion under the mild condition densified the matrix structure and improved the structure order to reduce the susceptibility to enzymatic breakdown. HA7-WHE, WHE, HA7-POT, and POT extrudates largely showed similar *in vitro* digestibility compared to their counterparts boiled in water. For all the five starch samples, the extrudates from the extreme condition generally possessed higher RDS contents but lower SDS/RS contents than those from the mild condition, indicating that the former samples had higher enzymatic digestibility. The extreme condition gelatinized the starch samples and degraded the molecules to a greater level as discussed in **Sections 4.4.2-4.4.4**, thus enhancing the enzymatic susceptibility. The determined SDS and RS contents of the starch blend extrudates were largely lower or similar to the corresponding values from calculation. The results suggested that the extrusion of starch blends did not further promote molecular interactions to enhance the enzymatic resistance.

Table 4.6 Starch digestibility of individual starch and starch-blend extrudates from mild and extreme conditions.[a,b]

	Mild			Extreme		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
HA7	54.9 ± 0.8 a	12.9 ± 1.1 b	27.4 ± 0.9 c	63.2 ± 1.0 a	10.7 ± 1.3 b	22.8 ± 1.1 d
HA7-WHE	70.2 ± 1.4 b	4.2 ± 1.2 a	19.6 ± 1.3 b	74.6 ± 0.6 b	5.1 ± 2.7 a	16.1 ± 2.4 c
	(70.4)[c]	(8.3)	(17.3)	(73.0)	(6.8)	(15.2)
WHE	85.8 ± 0.9 d	3.6 ± 1.8 a	7.2 ± 1.9 a	82.8 ± 2.2 c	2.9 ± 1.9 a	7.6 ± 0.8 a
HA7-POT	70.9 ± 0.7 b	3.3 ± 1.4 a	20.3 ± 1.4 b	76.6 ± 0.5 b	3.8 ± 0.6 a	14.2 ± 0.6 bc
	(68.5)	(8.0)	(18.6)	(73.0)	(6.8)	(17.4)
POT	82.1 ± 1.1 c	3.0 ± 2.6 a	9.7 ± 1.6 a	82.8 ± 1.9 c	2.8 ± 0.8 a	12.0 ± 1.5 b

[a]Values are presented as average ± standard deviation (n = 4); in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b]RDS: rapidly digestible starch, SDS: slowly digestible starch, and RS: resistant starch; values were calculated on dry basis.

[c]Theoretical %RDS/SDS/RS of starch-blend extrudate = (%RDS/SDS/RS of cooked extruded Starch A × 50%) + (%RDS/SDS/RS of cooked extruded Starch B × 50%).

4.5 Conclusions

The present study aimed to examine whether extrusion could further enhance the functional properties and enzymatic resistance of HA7-WHE and HA7-POT starch blends and how the extrusion conditions used determined such effects. The reduction in amylose contents of the starch samples after extrusion suggested that the processing could effectively degrade starch molecules, while the two processing conditions did not result in marked differences in the amylose contents of the starch samples, except for POT. The extreme condition (*i.e.,* high temperature and shear, and low moisture) led to a higher degree of starch gelatinization for all the starch samples. The contents of damaged starch/gelatinized starch of starch extrudates were generally between those of the corresponding extruded individual starches. Higher instant viscosities and lower viscosity development during pasting were observed for all the starch samples in comparison with their native counterparts, which could be ascribed to the starch gelatinization, loss of granular structure, and molecular degradation caused by the extrusion processing. Starch gelatinization and molecular degradation induced by extrusion diminished the ability of the starch blends and individual starches to develop firm gels after cooking and storage. HA7-WHE and HA7-POT extrudates largely showed similar *in vitro* digestibility when compared with their counterparts boiled in water, which suggested that the extrusion of starch blends did not further promote molecular interactions to enhance the enzymatic resistance. The new information presented in this research will provide insights for the food industry to produce novel pre-gelatinized starch ingredients for different food products.

5 GENERAL DISCUSSION

In the current work, we blended high-amylose maize starch (HA7) with wheat (WHE) or potato (POT) starch at 1:1 ratio (dwb). We then characterized the thermal properties, pasting and gelling behaviors at various heating temperatures, gel microstructure, and *in vitro* starch digestibility of the resulting starch blends along with the three individual native starches. Subsequently, we extruded the starch blends and the three native starches under different extrusion conditions to examine the role of granular structure in the new functional attributes observed in the native starch blends. Two sets of extrusion conditions were selected in second study: mild condition (*i.e.,* high feed moisture, low temperature, and low shear) and extreme condition (*i.e.,* low feed moisture, high temperature, and high shear).

We have demonstrated that although the gelatinization of individual starches in the starch blends appeared to be independent in the native starch blends, the HA7-WHE blend had exceptionally low percentage of retrogradation (9.2%) than the theoretical value (27.7%) when assuming simple addition (**Table 5.1**). The findings could be attributed to the formation of a large amount of amylose-lipid complexes (ALC) in HA7-WHE blend (**Table 5.1**), which are known to effectively inhibit the re-formation of double helices in gelatinized starch during storage. Since retrogradation is the main factor responsible for syneresis and other associated instability phenomena of cooked starch during storage, particularly under cold conditions (*e.g.*, 4°C), the significant decrease in retrogradation by simply blending HA7 and WHE could be used to develop native starch ingredients with enhanced storage stability. In contrast, the measured retrogradation value of HA7-POT was higher than expected (44.9% *versus* 32.9%).

Table 5.1 Enthalpy changes (ΔH) of dissociation of amylose-lipid complexes (ALC) and percentages of retrogradation of three native starches and starch blends at 1:1 ratio (dwb).[a,b]

	ΔH of ALC dissociation	%Retrogradation[c]
HA7	1.9 ± 0.1 b	44.8 ± 0.8 c
HA7-WHE	1.9 ± 0.2 b	9.2 ± 1.4 a
	(1.5)[d]	(27.7)[e]
WHE	1.0 ± 0.1 a	10.6 ± 0.5 a
HA7-POT	0.7 ± 0.0 a	44.9 ± 3.4 c
	(1.0)	(32.9)
POT	n.d.	20.9 ± 0.8 b

[a] Data in this table were adapted from **Tables 3.1** and **3.2**.

[b] Determined using a differential scanning calorimeter (DSC). Values are presented as average ± standard deviation (n = 3). The numbers with the same letter in a column are not significantly different at $p < 0.05$.

[c] The percentage of retrogradation was calculated in accordance with the following equation: %Retrogradation = (ΔH of melting of retrograded starch) / (ΔH of gelatinization of native starch) × 100%.

[d] Theoretical ΔH of ALC dissociation in starch blend = (ΔH in Starch A × 50%) + (ΔH in Starch B × 50%).

[e] Theoretical %Retrogradation of starch blend = (%Retrogradation of Starch A × 50%) + (%Retrogradation of Starch B × 50%).

Pasting behavior is one important functional attribute of starch ingredients that determines their end uses. We showed that the pasting profiles of the starch blends and their extrudates could not be simply explained by the proportional superposition of the pasting performance of the individual starches. In native HA7-POT blend, POT was the major contributor of viscosity development of the blend, and hence the pasting behavior of HA7-POT generally reflected that of POT. As the holding temperature increased from 95 to 140°C, the pasting temperature and the peak viscosity and time generally remained the same, while the trough and final viscosities gradually decreased, resulting from thermal degradation and thixotropic breakdown. By contrast, at all the three heating temperatures (95, 120, and 140°C), HA7-WHE blend exhibited the highest peak, trough, and final viscosities at 120°C. The different change patterns of the pasting profiles of HA7-WHE across the test heating temperatures could be related to the importance of HA7 for the viscosity development of the blends. It is noteworthy that the pasting viscosity of HA7-WHE was even higher than that of WHE alone between 12.9 and 29.6 min at 120°C heating (**Figure 5.1**, Native, highlighted region), which suggested that certain physical interactions occurred between the different components from those two starches to promote the viscosity development. It is critical to point out that the noted resistance to high-temperature pasting at 120°C diminished after the extrusion of HA7-WHE as demonstrated in **Figure 5.1,** Extruded, suggesting the importance of granular structure of starches for the unique feature of native HA7-WHE sample.

In Study 2, different levels of starch gelatinization and molecular degradation were observed for starches and starch blends after extrusion processing. The extreme extrusion condition induced more severe damage and gelatinization of the starches. Hence, compared with the native individual starches and starch blends, the extruded counterparts demonstrated higher viscosity development in the initial stage of pasting (*i.e.,* more obvious development of instant viscosity) and lower overall pasting viscosities in the later stage (**Figure 5.1** and **Table 5.2**). Among HA7, WHE, and POT, the applied extrusion processing led to the most apparent change in the pasting profile of POT, which could be related to the extensive swelling of POT granules upon thermal processing. POT extrudate from the mild extrusion condition was able to promptly develop viscosity, thus showing the shortest peak time and the highest peak viscosity over the holding temperatures of 95-140°C among all the extrudates obtained from this processing condition (**Table 5.2**). In contrast, the extreme extrusion condition caused severe gelatinization and molecular degradation of POT, and therefore, at the heating temperatures of 95-140°C, the

resulting POT extrudate exhibited considerably lower peak viscosity than those of WHE and HA7-POT extrudates from the same processing condition (**Table 5.2**).

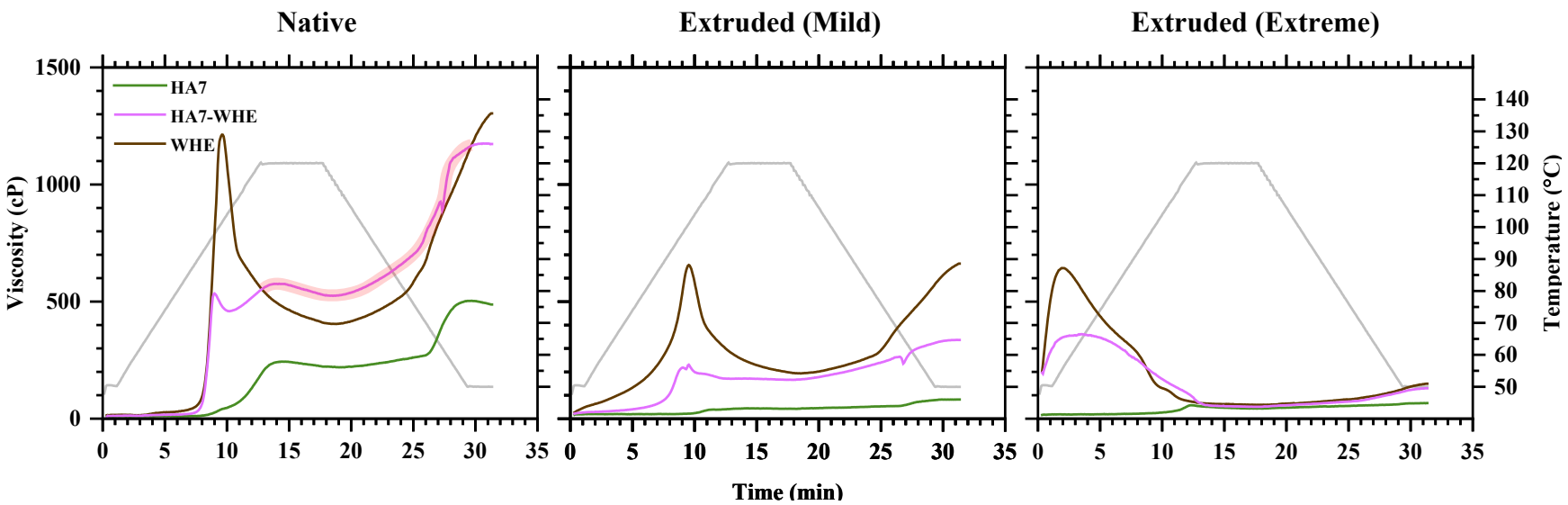


Figure 5.1 Pasting behaviors of native and extruded high-amylose maize starch (HA7), wheat (WHE), and their blend (HA7-WHE) at 1:1 ratio (dwb) analyzed using RVA 4800 at 120°C heating. Starch suspensions (28.0 g in total) containing 8.0% of dry solids were used for the analysis. The figure was adapted from **Figures 3.2** and **4.2**.

Table 5.2 Peak viscosity of native and extruded three starches and starch blends at 1:1 ratio (dwb) analyzed using RVA 4800 at heating temperatures of 95, 120, and 140°C.[a,b]

	Holding temperature (°C)	Peak viscosity (cP)		
		Native	Extruded	
			Mild	Extreme
HA7	95	40.5 ± 2.1	19.5 ± 1.3	23.8 ± 2.4
	120	208.5 ± 7.8	42.0 ± 2.6	57.5 ± 2.4
	140	230.0 ± 11.3	42.8 ± 3.3	55.0 ± 6.8
HA7-WHE	95	440.5 ± 2.1	270.3 ± 3.9	76.8 ± 4.6
	120	560.0 ± 12.7	230.8 ± 3.6	96.3 ± 4.4
	140	483.0 ± 11.3	229.5 ± 2.1	91.0 ± 3.6
WHE	95	1345.5 ± 2.1	745.0 ± 15.7	602.8 ± 22.4
	120	1213.0 ± 11.3	657.3 ± 27.1	583.3 ± 26.4
	140	1218.0 ± 19.8	687.8 ± 20.4	583.5 ± 27.1
HA7-POT	95	2404.0 ± 9.9	438.5 ± 24.3	358.8 ± 32.2
	120	2551.5 ± 23.3	428.0 ± 17.6	362.0 ± 9.6
	140	2550.5 ± 30.4	433.8 ± 27.7	376.3 ± 5.3
POT	95	9081.0 ± 198.0	2531.8 ± 44.7	131.8 ± 13.8
	120	8684.5 ± 422.1	2480.3 ± 215.7	147.8 ± 4.9
	140	8820.0 ± 144.2	2517.5 ± 71.0	128.3 ± 6.8

[a] Data in this table were adapted from **Tables 3.3** and **4.4**.

[b] Determined using a Rapid Visco Analyzer (RVA 4800), and 28.0 g of starch suspensions containing 8.0% of dry solids were used for the analysis. Values are presented as average ± standard deviation (n = 2).

For normal starches (*i.e.,* amylose content between 20% and 40%), swollen starch granules/remnants with good integrity are critical for gel formation after cooking and cooling. Such swollen starch granules/remnants can fill the space in the mold and develop a dense gel network, which showed as "cells" in the lyophilized gel matrix (*e.g.,* **Figure 3.3**, WHE – 95°C). However, as the heating temperature increased to 140°C, WHE granules/remnants gradually lost the integrity, and the starch molecules became uniformly dispersed to develop a flaky and loose matrix structure (*e.g.,* **Figure 3.3**, WHE – 140°C), which thus had reduced gel strength. On the other hand, the gelation mechanism of high-amylose starches is different from those of normal starches. Since amylose is the major component of high-amylose starches (> 50%), full dispersion of starch molecules is the key factor for linear amylose to re-associate with each other and support a compact gel matrix structure (*e.g.,* **Figure 3.3**, HA7 – 140°C). The described different gelation mechanisms explained the different change patterns of WHE and POT, and HA7 as the heating temperature increased from 95 to 140°C (**Table 5.3**). Although the gelatinization and molecular degradation induced by extrusion as described above disintegrated starch granules of HA7, WHE, and POT and decreased their gelling ability (**Table 5.3**), the change patterns of gel strength of HA7, WHE, and POT extrudates from both processing conditions were in general similar to those of their native counterparts over 95-140°C heating.

HA7-WHE blend cooked at 120°C and HA7-POT sample cooked at 95 and 120°C showed higher measured gel strength than their corresponding theoretical values (**Table 5.3**). The results suggested that the interactions between starch granules/remnants and molecules could help to maintain the gelling ability of the two starch blends at the indicated high heating temperatures. HA7-WHE extrudate from the mild condition displayed gel strength values slightly higher than the corresponding theoretical data after cooking at 95 and 120°C (**Table 5.3**). For HA7-WHE extrudate from the extreme condition and HA7-POT extrudates from both processing conditions, the determined gel strength data were consistently smaller than the calculated values at the three cooking temperatures. The weakened gelation capability of the starch-blend extrudates could be explained by the observation that the extrusion processing disrupted starch granular structure and degraded starch molecules.

Table 5.3 Gel strength of native and extruded starches and starch blends after cooking at 95, 120, and 140°C.[a,b,c]

	Holding temperature (°C)	Gel strength (g)		
		Native	Extruded	
			Mild	Extreme
HA7	95	8.5 ± 0.6 a	3.9 ± 0.8 a	9.9 ± 3.7 b
	120	108.3 ± 3.1 c	12.2 ± 1.3 a	36.7 ± 7.6 c
	140	604.3 ± 17.6 e	368.4 ± 29.2 e	295.6 ± 22.2 c
HA7-WHE	95	34.1 ± 1.9 b (42.2)[d]	32.9 ± 2.7 c (27.8)	3.9 ± 0.1 b (13.3)
	120	82.0 ± 1.1 c (77.2)	30.3 ± 2.9 c (17.1)	15.7 ± 1.0 b (21.3)
	140	109.4 ± 5.9 d (316.6)	94.1 ± 3.3 d (194.6)	50.1 ± 3.2 b (150.2)
WHE	95	75.9 ± 3.7 c	51.6 ± 8.7 d	16.7 ± 0.9 c
	120	46.1 ± 4.2 a	22.0 ± 0.8 b	5.8 ± 0.2 a
	140	28.9 ± 1.4 b	20.7 ± 0.4 b	4.8 ± 0.4 a
HA7-POT	95	33.3 ± 0.7 b (8.0)	4.8 ± 0.5 a (10.9)	3.5 ± 0.3 b (6.3)
	120	74.3 ± 3.5 c (56.5)	12.5 ± 1.0 a (13.7)	13.8 ± 2.8 b (19.6)
	140	78.4 ± 3.5 c (304.5)	53.9 ± 8.7 c (189.6)	46.1 ± 6.8 b (149.1)
POT	95	7.5 ± 0.2 a	17.9 ± 1.5 b	2.7 ± 0.1 a
	120	4.6 ± 0.1 b	15.1 ± 0.5 a	2.5 ± 0.1 a
	140	4.7 ± 0.2 a	10.7 ± 0.9 a	2.6 ± 0.0 a

[a] Data in this table were adapted from **Tables 3.4** and **4.5**.

[b] Starch or starch extrudate suspensions (28.0 g containing 8.0% of dry solids) were cooked following the same procedure used for the pasting property determination; the resultant pastes were poured into a screw-capped plastic canister and stored at ambient temperature for 2.0 h for gelation.

[c] Values are presented as average ± standard deviation; in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[d] Theoretical gel strength of starch blend = (Gel strength of native or extruded Starch A× 50%) + (Gel strength of native or extruded Starch B × 50%).

Starch ingredients with low digestibility can offer various benefits after consumption. In the thesis research, it was demonstrated that simple blending of HA7 with WHE or POT decreased the *in vitro* digestibility of the cooked native starch blends (**Table 5.4**). Particularly for cooked HA7-WHE blend, the determined RS content (69.7%) was noticeably lower than the theoretical value (75.4%), which could be partially associated with the formation of ALC in this sample. ALC is known to be type 5 RS (RS5) in the literature (Ai *et al.*, 2013).

Extrusion effectively gelatinized the starch samples and degraded the molecules as illustrated in Study 2, and thus the extruded starches generally showed high *in vitro* starch digestibility (**Table 5.4**). Interestingly, HA7 extruded under mild condition possessed considerably lower digestibility compared with its native counterpart boiled in water: RDS content of 54.9% in the former *versus* 63.0% in the latter, which could be attributed to the formation of densified matrix structure and improved structure order caused by the mild extrusion processing (Htoon *et al.*, 2009). However, the starch-blend extrudates largely showed *in vitro* digestibility similar to that of their counterparts cooked in boiling water, which suggested that the extrusion of starch blends did not further enhance the enzymatic resistance.

Table 5.4 Rapidly digestible starch (RDS) contents of native starches and starch blends after cooking in boiling water and their respective extrudates without further boiling in water.[a,b]

	RDS (%)		
	Native	Extruded	
		Mild	Extreme
HA7	63.0 ± 1.5 a	54.9 ± 0.8 a	63.2 ± 1.0 a
HA7-WHE	69.7 ± 0.3 b	70.2 ± 1.4 b	74.6 ± 0.6 b
	(75.4)[c]	(70.4)[d]	(73.0)
WHE	87.8 ± 1.4 d	85.8 ± 0.9 d	82.8 ± 2.2 c
HA7-POT	70.5 ± 2.6 b	70.9 ± 0.7 b	76.6 ± 0.5 b
	(72.8)	(68.5)	(73.0)
POT	82.6 ± 1.9 c	82.1 ± 1.1 c	82.8 ± 1.9 c

[a] Data in this table was adapted from **Tables 3.5** and **4.6**.

[b] Values are presented as average ± standard deviation; in the same column, the numbers with the same letter are not significantly different at $p < 0.05$.

[b] Theoretical %RDS of cooked starch blend = (%RDS of cooked Starch A × 50%) + (%RDS of cooked Starch B × 50%).

[d] Theoretical %RDS of cooked starch-blend extrudate = (%RDS of extruded Starch A × 50%) + (%RDS of extruded Starch B × 50%).

6 GENERAL CONCLUSIONS AND FUTURE STUDIES

The overarching goal of the present research was to develop new "clean-label" starch ingredients with diversified functional properties and improved nutritional profiles. High-amylose maize starch (HA7) was blended with wheat (WHE) or potato (POT) starch at 1:1 ratio (dsb), and then the thermal properties, pasting and gelling behaviors over 95-140°C heating, and *in vitro* digestibility of HA7-WHE and HA7-POT blends and three individual starches were characterized. The gelatinization of individual starches in the blends appeared to be independent. Interestingly, the formation of a large amount of amylose-lipid complexes (ALC) was observed in HA7-WHE blend but not in HA7-POT blend. Leached-out amylose molecules from HA7 might complex with the surface endogenous lipids of WHE, which accounted for the large amount of ALC in HA7-WHE blend. The large amount of ALC in HA7-WHE resulted in a low percentage of retrogradation of HA7-WHE blend in cold storage at 4°C, which suggested that blending of HA7 and WHE could be a promising approach to improve the cold-storage stability of starch ingredients. The starch blends also demonstrated good resistance to thermal degradation and thixotropic breakdown during pasting at 120°C, suggesting that certain physical interactions between the components of different starches promoted viscosity development at the high heating temperature. Therefore, these blends could be used in food products that undergo harsh processing conditions, such as canning, retorting, and jet-cooking. With respect to the gel strength of starch blends, the measured gel strength of HA7-POT blends after cooking at 95°C was higher than the theoretical value when assuming simple additivity, which might be ascribed to that the diffused-out amylose molecules from HA7 maintained the granular stability of POT as revealed by scanning electron microscopy (SEM). After cooking at 120°C, HA7-WHE and HA7-POT blends formed gels stronger than anticipated, which is in good agreement with the pasting profiles of the starch blend samples. The determined resistant starch (RS) and slowly digestible starch (SDS) contents of water-boiled HA7-WHE blend were higher than the theoretical values, indicating the reduced digestibility of the starches through simple blending.

In order to understand if extrusion could further diversify the functional profiles and *in vitro* digestibility of the same starch blends and how the different extrusion conditions determine such effects, we extruded the same starch blends and three individual starches under two sets of extrusion conditions in Study II: mild condition (high feed moisture, low barrel temperature, and low screw speed) and extreme condition (low feed moisture, high barrel temperature, and screw speed). The functional attributes and *in vitro* digestibility of the starch extrudates were characterized and compared with those of their native counterparts. Extruded starch blends and starches showed decreased amylose contents in comparison with their native counterparts, while the mild and extreme conditions showed similar reducing effects on the amylose contents of the starch samples, except for POT and HA7-POT blend. The reduction in starch amylose contents after extrusion suggested that the processing was able to degrade amylose molecules (most likely amylopectin too). As expected, higher barrel temperature and more shear in extreme condition could degrade and/or gelatinize starches to greater extents. Regarding the pasting properties, the starch extrudates were able to develop viscosities at low temperature (except for HA7 extrudates), but their pasting viscosities were generally lower than those of their respective native samples, which could be attributed to starch gelatinization, loss of granular structure, and molecular degradation resulting from extrusion. The aforementioned changes induced by extrusion also diminished the ability of starch to form strong gels. Additionally, similar *in vitro* digestibility profiles were observed for the extrudates of starch blends and individual starches when compared with their water-boiled unextruded counterparts, which suggested that the extrusion of starch blends did not further promote molecular interactions to enhance the enzymatic resistance.

The new information presented in current work will be useful for the food industry to develop native and pre-gelatinized starch ingredients with desirable functionalities and improved nutrition profiles that can be utilized to prepare high-quality and nutritious food products. Future research can include, but not limited to: (1) Investigating the functional properties and *in vitro* digestibility of defatted HA7-WHE and HA7-POT blends to further examine the influence of ALC formation on the reported techno-functional attributes of the two starch blend samples; (2) Evaluating the performance of the prepared native and extruded starch blend samples in different food products; and (3) Determining the effects of other physical modifications, such as heat-moisture treatment, high hydrostatic-pressure treatment, and radiation, on the physicochemical properties and *in vitro* digestibility of HA7-WHE and HA7-POT blends.

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Appendix A

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Figure 2.2

Caption:

(a) Proposed molecular structure of amylose, where EL = extra long chain (DP > 2730), L = long chain (DP > 230), S = short chain (DP ~18), and Ø = reducing end. (b) Cluster model of amylopectin, where C.L. = chain length, and Ø = reducing end

Source:

Ai, Y., & Jane, J. (2018). Understanding starch structure and functionality. In M. Sjöö & L. Nilsson (Eds.), *Starch in Food* (2nd ed., pp. 151-178): Woodhead Publishing.

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Figure 2.3

Caption:

Molecular arrangement in the crystalline structure of starch: a) double-helical crystallite (side view) formed between two starch chains; b) monoclinic organization (top view) of double-helical crystallites in starch with the A-type X-ray diffraction pattern; c) hexagonal organization (top view) of double-helical crystallites of starch with the B-type X-ray diffraction pattern; d) A-, B-, and C-type X-ray diffraction patterns of starch. In (b) and (c), black dots (•) represent water molecules, black lines (──) represent covalent bonds, and dashed lines (----) represent hydrogen bonds.

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Figure 2.7

Caption:

Pasting behaviors of wheat (a) and maize (b) starch before and after washing with 2% (w/v) sodium dodecyl sulfate (SDS), a detergent that can effectively remove the endogenous lipids and the surface proteins in starch granules.

Source:

Debet, M. R., & Gidley, M. J. (2006). Three classes of starch granule swelling: Influence of surface proteins and lipids. *Carbohydrate Polymers, 64*(3), 452-465.

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